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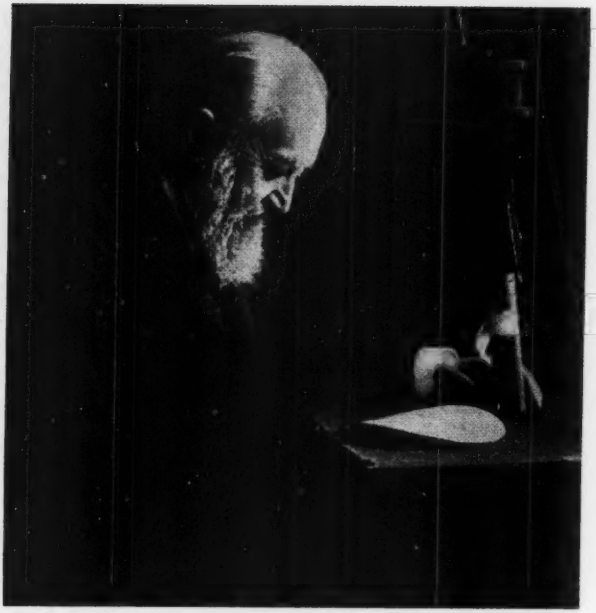

Dedicated to the memory of
EWALD HERING
1834 - 1918

Gemischtes Licht erscheint farblos, wenn es sowohl für die blaugelbe als für die rotgrüne Substanz ein gleichstarkes Dissimilirungs- wie Assimilirungsmoment setzt, weil dann beide Momente sich gegenseitig aufheben, und die Wirkung auf die schwarzweisse Substanz rein hervortritt.

HERING, E.: Zur Lehre vom Lichtsinne. Wien 1878. pp. 120—121.

I. A TECHNIQUE FOR OSCILLOGRAPHIC RECORDING OF SPECTRAL RESPONSE CURVES

BY

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In an earlier paper (*Svaetichin* 1953 a) microelectrode experiments were described, in which a membrane action potential was recorded from the cone layer of the fish retina in response to light. Further experiments of the same type have now been performed, and the present paper gives an account of the physical and biological methods used in these investigations.

Four micromanipulators of the Zeiss sliding type were mounted in conjunction with a Leitz Laborlux microscope, on the stage of which a Zeiss dissecting microscope could be easily interchanged with the Laborlux as required. The Laborlux was equipped with Newton long working distance objectives for use when a higher resolution was required. The complete assembly of microscope and micromanipulators was mounted on an aluminium plate which was supported by a lead filled pillarstand (see Fig. 1).

The potentials were led off with high impedance electrodes which fed into a frequency compensated cathode follower input stage (*Haapanen* & *Ottoson* 1954) and all the recordings were made with D. C. amplifiers (Offner Electronics type 142 or Tektronix plug-in unit type 53D/54D, the output being connected to the vertical deflection circuit of a Tektronix oscilloscope (type 545).

In most experiments we used a photostimulator built on an optic bench which rested on a heavy concrete table (see Fig. 1). The light source, which was mounted in an air cooled lamp base, was a 6 V, 100 W wolfram filament lamp (Philips type 13105B) with a filament coil having a diameter and length of 3 mm. The filament was fed from the 60 c/sec A.C. mains, the voltage of which was stabilized to less than 0.5 %.

When measured with a photocell, the "ripple" of the light due to the A. C. heating of the filament, was found to be about 5 %. This small value was due to the large thermal capacity of the filament coil. As could be expected, the effect of this ripple was undetectable on the records from the vertebrate or insect eyes.

All the lenses used were antireflection coated symmetrical aplanats. From the collimator lens the parallel light beam passed through a

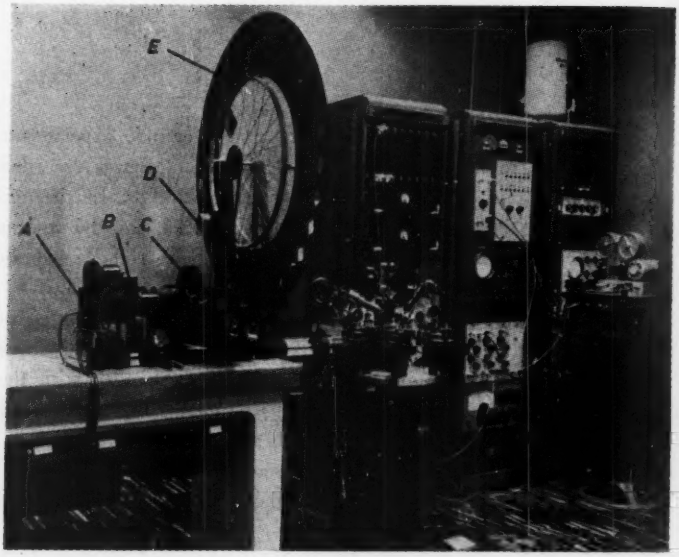

Fig. 1. The photostimulator can be seen to the left of the photograph; in the centre the microscope-micromanipulator assembly, while to the right stands the electronic recording equipment. A indicates the light source; B the shutter mechanism; C the neutral-density filter wheels; D the sweep potentiometer which is mechanically connected to the interference filter wheel E.

stable filter, which to some extent absorbed red and infra-red, and also through an aperture diaphragm. With the aid of additional lenses, the image was adjusted to be a little out of focus, and was projected onto the preparation as a light spot of uniform intensity. For a rough change of light stimulus intensity an iris diaphragm was used. In order to obtain a more accurate variation in the light intensity, a set of 5×5 cm neutral density filters was used (Bausch & Lomb Optical Co.), which covered a density range of up to 6 Log units in 0.3 steps and also a neutral density wedge (5×15 cm) with a density range of 0—1. The neutral density filters were mounted on two wheels interconnected by gears in such a way that by rotating one wheel slowly by hand or from an electric motor via a reduction drive, a range of densities from 6.0—0 in 0.3 steps was sequentially obtained. A linear potentiometer

with associated battery was coupled to this device in such a way that when the potential tapped off was applied to the horizontal deflection circuit of the oscilloscope, the cathode ray beam deflection was made proportional to the total density of the filters in the light beam. At the extreme left the spot deflection represented the density 6.0, and at the extreme right the density zero. This is shown in the photographed scale of Fig. 2 B. This scale had been engraved on a plexiglass plate placed in front of the screen of the cathode ray tube. Light entering this plate from its edge, produced a scattering illumination of the engraved scale.

All the recordings were made with a Grass camera, which was fitted with an automatic single sweep numbering device.

When the light beam struck the centre of the filters for any of the possible 21 combinations, a microswitch was operated, thereby electronically energizing the electromagnet of the shutter mechanism for a pre-determined period, thus enabling a rectangular pulse of light of controllable duration to reach the preparation.

The tracing of 21 consecutive responses to light stimuli of increasing intensity, as indicated on the Log scale below in Fig. 2 B, shows the relation between the amplitude of the photoreceptor response and the intensity of the light stimulus used. Thus, the response together with the scale as shown in Fig. 2 B was conveniently obtained on a single photographic record.

In order to produce narrow bands of spectral light from the visible part of the spectrum, interference filters were used (Geraetebauanstalt Balzers, type Filtraflex B). The peak values given in mμ of the transmission curves of the 24 (5$\times$5 cm) filters used, were as follows: 401, 427, 440, 453, 462, 472, 487, 502, 511, 518, 535, 547, 557, 572, 586, 600, 616, 630, 642, 654, 668, 689, 717, 750. These filters were mounted close to the periphery of a 110 cm diameter plexiglass plate, which in turn was fixed to a bicycle wheel supported by its ball bearings. The angular distance between the filters on the assembly was made proportional to their wavelength difference. The wheel with its set of filters was mounted on the optic bench in such a way that the beam of light entering the interference filter had an angle of incidence and convergence well below 5°; in this manner each filter could easily be placed in the path of the light bundle. Only a restricted area in the centre of each filter (2 cm^2) was generally used for transmitting light.

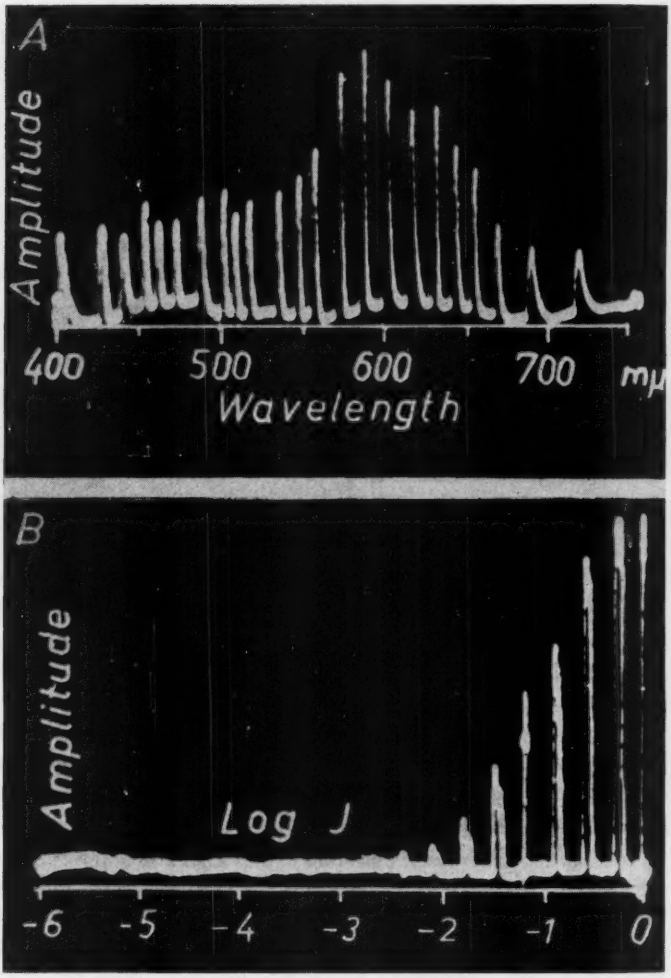

Fig. 2. A shows the spectral response curve recorded from a cone by the aid of a microelectrode. B shows the relation between response amplitude and Log intensity of the colorless light stimulus as recorded from the same cone.

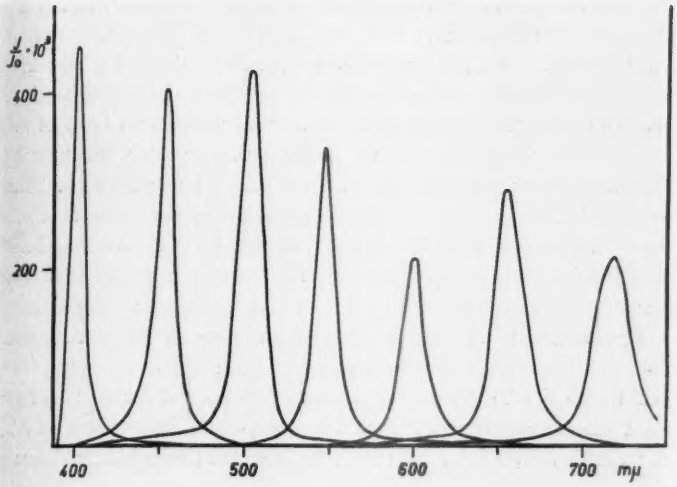

Fig. 3. Transmission curves for seven of the interference filters used in these studies (total amount 24).

The axle of the wheel was joined to the shaft of a linear potentiometer in order to obtain the oscilloscope horizontal deflection voltage in a manner similar to that described earlier. Thus, the oscilloscope horizontal axis could be calibrated in wavelength, and an engraved scale illuminated by scattered light was used for this purpose. When the beam of light struck the centre of a filter, a microswitch was closed, thus operating the previously mentioned shutter mechanism. The stated peak values of the interference filters were accurate to within $\pm$ 2 mμ with half value widths of 12—20 mμ. Transmission curves obtained by measurements in the laboratory agree well with curves provided by the manufacturer. Fig. 3 shows a set of the transmission curves obtained from some of the interference filters used.

All of the mounted interference filters were matched by the aid of neutral density filters; this ensured that the light stimulus from each contained an equal amount of energy. The neutral density filters used for this purpose were made of superannuated fine grain film, while the

final minute energy differences were adjusted with the aid of 2 mm thick plates of plexiglass.

The energy of the light transmitted through the filters was measured in the normal position of the retinal preparation by means of a microthermocouple. The thermocouple was covered with a thin layer of mat black lacquer, which in turn was coated with lampblack. The thermocouple was enclosed in an airtight space with a 1 mm thick plexiglass window in front of the junction. By using the thermocouple as a zero recording instrument the transmitted energy from the various interference filters was matched with an accuracy of better than 3 %. This energy equality was checked from time to time; and in particular when a new source lamp was fitted, a small adjustment of the filament voltage was usually required. A voltage drop across the filament from 6 V to 5.5 V (8.5 %) caused an energy drop of about 28 % of the light transmitted through the 400 mμ filter, and a drop of about 17 % for the 717 mμ filter as measured with the thermocouple. Thus a voltage drop of 8.5 % produced an energy difference of about 15 % for the light transmitted through the two filters situated at the extreme ends of the spectrum under investigation. However, the energy change of 15 % would approximately correspond to a difference of only 1—2 % of the photoreceptor response amplitudes (see Fig. 2 B). As previously mentioned, the filament voltage was stabilized to better than 0.5 %. Also a close examination of the image used for stimulating the retinal preparation revealed that no chromatic aberration was introduced by the optical system over the range of wavelengths used.

With this apparatus, records showing the photoreceptor potentials as a function of wavelength (the spectral response curve) and the potential as a function of light intensity (the amplitude-intensity relation curve), could be obtained within about 60 seconds total recording time. Therefore, the records were usually exactly reproduceable, the functional state of the preparation not having enough time to change appreciably between the consecutive series of measurements. This is an important point, in view of the fact that the time available for measurements after puncturing the photoreceptor with the microelectrode is limited, being only from about 5 to 15 minutes.

In Fig. 2 A one of the spectral response curve recordings from a single cone is shown, while Fig. 2 B shows the amplitude intensity relation curve for colorless light for the same photoreceptor. The wave-

Fig. 4. The exposed cone layer of the fish eye as seen under the dissecting microscope. Notice the regular rows of cones.

length and intensity scales are included in the record photograph of the oscilloscope screen.

Selected healthy fish *(Teleost)* caught not more than two days before an experiment and kept in an aquarium, were used for the present investigation. The fish was decapitated and the eye carefully removed without subjecting it to any pressure. The sclera and the chorioid of the posterior half-sphere of the eye were then removed with the aid of microscissors and microforceps. This operation lasted about five minutes. The preparation was then put into a moist chamber containing moist saturated oxygen (or O_2+5 % CO_2).

Under the dissecting microscope the pigment layer together with the adhering outer parts of the rods were simply lifted away. If the animal

had previously been adapted to light, this removal was an easy procedure, although it only succeeded completely in the inferior half of the retina, which appears to function as a central area for day light vision (*cf. Wunder* 1925).

Upon illuminating the retina by transmitted light through its own lens system, one could see the regular rows of cones. The micrograph in Fig. 4 shows the exposed cone layer of the fish retina as seen through the dissecting microscope.

Since any kind of mechanical interference or "scraping" was carefully avoided, examination of the prepared retina under high power microscope revealed no sign of damage to the cones. Moreover, as a result of the process of light adaption the rods could be cleanly removed with the layer of pigment cells into which they had migrated. In this procedure the very thin (0.5 μ) rod myoids break (see *e.g. Walls* 1942, Fig. 170, and *Svaetichin* 1956, Fig. 7). Since the cones were exposed and well oxygenated, their electrical response to light increased. If the eye had previously been well dark adapted, it was possible to remove the pigment layer alone, thus leaving behind the rods intact as well. From such a preparation not only the cone activity but also the response of the rods could be recorded (*i.e.* the b-wave, see *Svaetichin* 1953 b). However, if the receptor layer was left unexposed, the b-wave rapidly disappeared, and soon the eye produced a slow, mainly negative ERG of small amplitude.

The preparation used in these experiments was in no case perfused with any artificial salt solution, since this had been found to reduce the survival time of the receptors. However, in order to reduce the effects of any possible receptor deterioration, the retinal preparation was not used for longer than 30—60 minutes. Eyes producing a poor electrical response to light were also rejected.

Concerning this retinal preparation which has previously been used (*Svaetichin* 1953 a), *Granit* (1955 p. 190) mentions that it consists of: "the receptors left over after removal of the pigment" that "has been scraped away", and that it "would seem more natural to study the cone action potential in a cone eye rather than in a damaged mixed eye". In fact, the preparation with an exposed receptor layer seems to be the only one possible to use when working with microelectrodes on single receptors, and as appears from the description given, the cones are obviously not damaged. For some reason (see *Svaetichin* 1953 b) the receptors in the fish eye (contrary to those of *e.g.* the frog) very soon suffer from oxygen lack if the receptor layer has not been exposed. Thus, the fish eye preparation described above seems to be one that has a considerable survival time.

Working on the fish eye with an unexposed receptor layer, *Granit* himself (1941) observed the short survival time but was not able to avoid it in his experiments. *Granit* (1941 p. 336) says: "Fish retinae are not very satisfactory material as the locus under the electrode quickly dies or loses in sensitivity". *Granit's* criticism of the above mentioned preparation with the exposed well oxygenated receptor layer is entirely unjustified.

The existence of "pure cone eyes" is rather uncertain and not easy to decide on a morphological basis (*e.g. Walls* 1942, *Prince* 1956), whereas from the ERG findings to conclude rod eyes appear to exist, *i.e.* eyes showing no a-wave but a b-wave and possibly a small negative "off" effect (*Parry, Tansley & Thompson* 1953, *Svaetichin* 1953 b). Thus, a less natural but more certain way to obtain a pure cone eye, is actually to remove the rods as described above.

KCL filled capillary microelectrodes were used of the type described by *Ling & Gerard* (1949) and *Nastuk & Hodgkin* (1950). The electrodes were automatically pulled by a device similar to that described by *Alexander & Nastuk* (1953). Electrodes produced by this method proved to be of a uniformly good quality, having a tip diameter smaller than 0.1 μ and a D. C. resistance somewhere between 20 and 100 MΩ. The electrodes were filled by capillary action in order to reduce a disturbing tip junction potential caused by boiling. Only electrodes selected shortly after filling were used.

It has been suggested (*Granit* 1955, p. 190) that the 20—30 mV positive contact potential, which appears when the electrode tip and cone membrane are in close juxta-position (*Svaetichin* 1953 a), is caused by the input tube grid current. This appears unlikely since the grid current taken by the selected 954 input tube was only 10^{-10} A. Hence, a total grid resistance change of about 30.000 MΩ would be required to produce a potential change of the same order as the contact potential. With a resistance of this order of magnitude in the grid circuit, the amplifier would be unstable and extremely sensitive to external disturbances. No such instability or undue sensitivity has been observed.

By feeding a positive rectangular pulse via a normally non-conducting diode into the input grid, the effect of any change of grid to earth resistance could be observed by examining the output waveform of the amplifier (*Haapanen & Ottoson* 1954). For example, if the grid resistance was to increase considerably, the shunting effect of the grid to earth capacitance would be increased, with a marked effect on the waveform of the rectangular pulse from the amplifier output. Such a marked increase in grid resistance has not been observed in these experiments. Using this method, only small changes of grid to earth resistance were observed after the electrode entered the retina. It should be noted that in order to observe contact potentials, the diameter of the electrode tip must be smaller than 0.1 μ. In this case we are apparently dealing with an electric surface phenomenon, possibly the zeta potential, distinctive from the electrode artefact reported by *Adrian* (1956).

As shown in Fig. 4, the exposed cone layer showed up quite clearly under the dissecting microscope, the retina being illuminated by the rather weak colorless light passing through its own lens.

As might be expected, it proved difficult optically to locate the exact position of the microelectrode tip as it approached the retinal surface; however, the arrival of the tip into the thin fluid surface layer of the cones was revealed by signals from both the oscilloscope and loudspeaker. The actual position of the electrode tip was shown by the micrometer gauge fitted to the micro-manipulator; this could be read to an accuracy of $\pm$ 1μ.

The microelectrode was then moved nearer to the cones, and at a depth of about 30 μ below the fluid surface a negative potential instantaneously appeared, lasting only for a few seconds. No further potential was evoked by stimulating the retina with light, and it seems plausible to suggest that the tip had entered the outer segment of a cone. At a depth of about 70 μ below the fluid surface a negative potential of about 40 mV instantaneously appeared, this value generally remaining constant for a period of 5 to 15 minutes. Upon stimulating the eye with light, keeping the electrode in this position, an electrical response with an amplitude of about 20 mV was obtained. It appears in this case that the tip had entered into the ellipsoid or myoid of a single cone (*e.g. Svaetichin* 1953 a).

If the microelectrode was moved further into the retina, a steady negative potential appeared. This potential was found to be independent of any light stimulus, from which it may be deduced that the electrode had penetrated the external limiting membrane. Any further advance of the electrode generally gave intra- or extracellular recordings of impulses from nerve cells.

Under the experimental conditions described above, the exposed cones were covered by a 30 μ fluid film. This film did not appear to absorb any light over the range of wavelengths used for stimulating the retina in these experiments. Also the absorption caused by the possible presence of blood and other pigments was negligible.

At a depth of 70 μ below the fluid film, two types of retinal elements were found: (1) broken inner parts of below 0.5 μ diameter rod myoids, and (2) large 8—10 μ diameter intact cones. Since the electrode tip diameter was smaller than 0.1 μ, the possibility of inserting this tip into more than one cone at a time was very remote.

The experimental findings obtained using the above technique, support the view that the electrical response to light is obtained via an electrode whose tip has punctured the ellipsoid or myoid of a single cone (*Svaetichin* 1953 a).

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REFERENCES

Adrian, R. H., J. Physiol. 1956. *133*. 631.

Alexander, J. T. & W. L. Nastuk, Rev. sci. Instrum. 1953. *24*. 528.

Granit, R., Acta Physiol. Scand. 1941. *2*. 334.

— Receptors and Sensory Perception. Yale Univ. Press. 1955.

Haapanen, L. & D. Ottoson, Acta Physiol. Scand. 1954. *32*. 271.

Ling, G. & R. W. Gerard, J. cell. comp. Physiol. 1949. *34*. 383.

Nastuk, W. L. & A. L. Hodgkin, J. cell. comp. Physiol. 1950. *35*. 39.

Parry, H. B., K. Tansley & L. C. Thompson, J. Physiol. 1953. *120*. 28.

Prince, J. H., Comparative anatomy of the Eye. Springfield 1956.

Svaetichin, G., Acta Physiol. Scand. 1953 a. *29*. Suppl. 106. 565.

— Acta Physiol. Scand. 1953 b. *29*. Suppl. 106. 601.

— Acta Physiol. Scand. 1956. This Supplement.

Walls, G. L., The Vertebrate Eye and its Adaptive Radiations. Michigan 1942.

Wunder, W., Z. vergl. Physiol. 1925. *3*. 1.

II. SPECTRAL RESPONSE CURVES FROM SINGLE CONES

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The main results of the present study were verbally presented at the XXth International Physiological Congress in Brussels, July 20—August 4, 1956. *Cf.* the written report by Motokawa at the same congress.

The *Young—Helmholtz* trichromatic theory of color vision (*Young* 1807, *v. Helmholtz* 1852, 1853, 1856, 1911), which is still the prevailing one, is based primarily on the fact that in the human eye a sensation of achromaticity can be produced by a proper mixture of three spectral colors. Although the famous color equations have put color mixing on a quantitative basis, they do not offer any conclusive proof of the existence of three different chromoreceptor mechanisms. In fact, several psychophysical observations are easier to explain in terms of a modified *Hering* opponent color theory (*Hering* 1876, 1925, *Müller* 1896, 1897, *v. Tschermak—Seysenegg* 1929, 1947, 1952 and *Linksz* 1952). Fundamental response curves, which clearly support a four color theory, have been determined with *Motokawa*'s method (*Motokawa* 1949 a, b, *Motokawa* & *Isobe* 1955), which is based on the effect a previous spectral light stimulus has on the threshold of an electric phosphene.

The electrophysiological approach to color vision studies based on microelectrode recordings from single retinal ganglion cells, initiated by *Granit* and *Svaetichin* (1939) on the frog, and continued by *Granit* mainly on the cat, led *Granit* (1943, 1945, 1947, 1955) to formulate his dominator—modulator theory on a trichromatic basis.

The spike activity recorded from single neurons in the retinal ganglion cell layer is governed by several cones and additional effects from the rods cannot be excluded. It is therefore difficult to come to any definite conclusion as to the bearing of this work on the problem of color vision on the basis of the data hitherto obtained from studies on retinal ganglion cells.

In his attempt to present *Hering*'s opponent color theory in a form compatible with present neurophysiological knowledge, *Linksz* (1952) postulated the existence of "twin wavelength discriminators" consisting of two pairs of mutually exclusive receptor mechanisms: one for red & green, and another for yellow & blue. In addition to the wavelength discriminators, he proposed a single "signaler type" of receptor mechanism corresponding to *Hering*'s luminosity (black—white) mechanism (*cf. Müller* 1897).

In fact, morphologically both double and single cones exist in the retina of most vertebrates, such as amphibians, reptiles, turtles, and birds. The number of double cones in relation to single cones in the retina is higher in diurnal species than in noctural ones (*e.g. Walls* 1942). The *Teleost* fish have "twin cones" and they represent the most common type of cone in the retina. The ratio of twin cones to single cones is higher in surface fish than in those living under dark conditions in the depths (*Wunder* 1925). The twin cones of fish consist of two identical cones fused together in the myoid and ellipsoid region, the outer segments being well separated (Fig. 7). The double cones of other vertebrates are composed of two similarly fused cones which are morphologically very different (*e.g. Walls* 1942, *Prince* 1956).

The existence of color vision in *Teleost* fish, which greatly resembles that in man, showing the color circle phenomenon, the complementary color rule and four hue discrimination maxima, has been convincingly demonstrated (*Bauer* 1910, v. *Frish* 1913, 1925, *Hamburger* 1926, and *Wolff* 1925, see the survey by *Walls* 1942). As well as in the fish, color vision has been proved to exist in reptiles, turtles and birds, *i.e.* in vertebrates possessing double or twin cones. Consequently, there might be a relation between the morphologic occurrence of double or twin cones and the presence of color vision. *Walls* (1942, pp. 60 and 585), who devoted much attention to the question of double and twin cones, stated: "The most that can be said is that the number of double cones relative to the number of cones tends to be high in strongly diurnal animals and low in strongly nocturnal ones", and "Clearly, the twin cone is associated with exposure to bright light".

With the exception of the primates, color vision appears to be absent in mammals, a vertebrate group in which the double cones are also lacking (*Monotremes* and *Marsupials* have double cones) (*e.g. Walls* 1942, 1953, *Prince* 1956).

As far as mammals are concerned, *Walls* (1942) suggests that during their evolution they have spoiled a color vision mechanism already perfected by their ancestors, and that the primates were forced to develop it anew. Thus the idea is not very remote that the wavelength discriminative cones of the primates in some way function in pairs (*cf. Linksz* 1952).

Since the fish cones are the largest in size of their kind, they offer a possibility of making intracellular recordings. It was this characteristic

together with the presence of color vision that determined my choice of the fish as experimental animal. With a view to reaching the single cone, I made intracellular recordings on the fish retina (*Svaetichin* 1953 a). These preliminary findings intimate the existence of different cones in fish with respect to their spectral response curves. In the present study further experiments have been made on the same lines, using a more refined technique.

The present paper comprises an analysis of the different spectral response curves obtained from fish cones. The peripheral mechanism, on which hue discrimination is based, is believed to be located in the outer segments of the cones. Consequently, it seems justified to infer that the spectral response curves presented in the following may contribute to elucidating some aspects of the problem of color vision in particular, and of the cone vision in general.

RESULTS

The methods used in the present study are described in earlier papers (*Svaetichin* 1953 a, b, and *Svaetichin* & *Jonasson* 1956). The experiments were performed on various species of *Teleost* fish, but the results presented here are those obtained from investigations on fish of the *Mugil* genus found in river mouths on the Venezuelan coast.

The records in Fig. 1 show the different types of spectral response curve obtained by inserting a microelectrode (tip diameter less than 0.1 μ) into a single cone. In order to elicit a response to a light stimulus with the amplification used in these studies, it was necessary to introduce the microelectrode through the cell membrane of the cone (myoid or ellipsoid) and to obtain a constantly negative resting potential. Each of the 12 spectral response curves presented in Fig. 1 is a direct photograph of tracings on the cathode-ray tube screen, while the simultaneously photographed scale at the bottom of each record gives the wavelength in mμ along the abscissa. The spectral response curves were obtained by stimulating the retinal preparation with 24 successive, nearly monochromatic light pulses of different wavelengths but of equal energy content and duration (200 msec). The transmission maxima in mμ of the interference filters used in these experiments were as follows: 401, 427, 440, 453, 462, 472, 487, 502, 511, 518, 535, 547, 557, 572, 586, 600, 616, 630, 642, 654, 668, 689, 717, 750.

Fig. 1. Types of spectral response curves recorded from fish cones.

Actually, the recordings in Fig. 1 only display the amplitude and polarity of the cone responses elicited by the subsequent spectral stimuli. The wave form of this electric response is generally a rectangular pulse of approximately the same duration as the stimulus (*Svaetichin* 1953 a). With the present recording technique the width of the trace is merely that of the beam spot diameter, although a minute sweep movement also occurred in the course of each cone response to a spectral stimulus, making it possible to differentiate between the "on" and "off" responses appearing in the recordings close to the neutral point (see below and Figs. 1, 4, and 5).

In these experiments it was found that three fundamentally different types of spectral response curves occur; they are indicated in Fig. 1 by L, R - G and Y - B. The spectral response curves shown in the second and third rows of Fig. 1 have been denoted R - G and Y - B, since their maxima appear approximately in the red & green and yellow & blue regions of the spectrum respectively. The type of spectral response curve presented in the first row has been named L, since it seems to correspond well to the luminosity mechanism.

A D.C. amplifier was used for all the recordings with exception of the three upper spectral response curves seen in row R - G of Fig. 1. A disturbing drift of the D. C. level in the long wave end of the spectrum is seen in the bottom record of the R - G row in Fig. 1. Another artefact in the same row caused by the time constant of the A. C. amplifier (used only in these three recordings) disturbs the zero level of the three upper records.

The two spectral response curves on the top of each vertical row represent subsequent recordings with the electrode in an unaltered position, whereas the two recordings below were made from other cones.

The L type curve had a mean maximum response at the wavelength of 574 mμ. The response to some of the spectral light stimuli used (400—750 mμ) or to colorless light was always a hyperpolarization potential, *i.e.* increased intracellular negativity. Five definite submaxima, situated at about 420, 453, 502, 630, and 717 mμ, appeared regularly in the recordings of spectral response curves of the L type.

The outlines of two individual spectral response curves of the L type have been drawn in Fig. 2. The response amplitudes (filled and open circles, ordinates) are given in per cent of the maximal one obtained

Fig. 1. Types of spectral response curves recorded from fish cones.

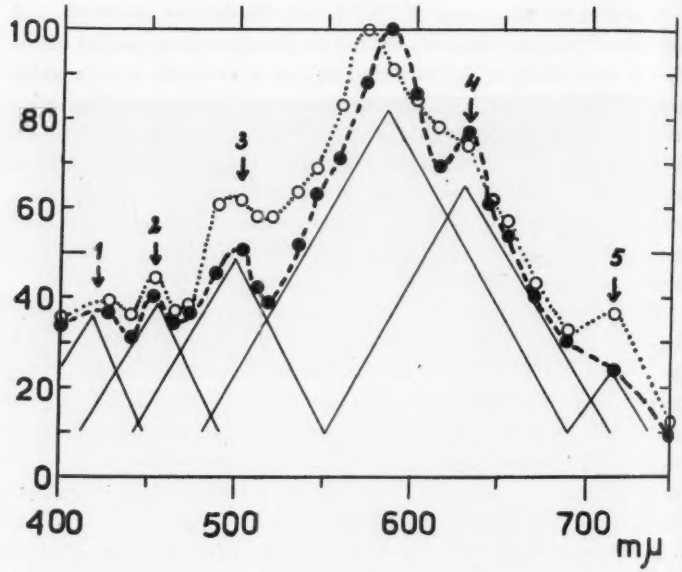

Fig. 2. Outlines of the L type spectral response curves. Continuous straight lines tentatively indicate suggested subcomponents which the L type of spectral response curve (broken lines) is composed of.

and plotted against the wavelength (abscissae). The curves show the five definite submaxima marked 1—5. The continuous lines denote suggested component curves, which the L type of spectral response curve (broken line) appears to be composed of.

The R-G type of spectral response curve (Fig. 1) disclosed a depolarizing response at the shorter wavelengths with a mean maximum value at 506 mμ; at longer wavelengths the electric response underwent a polarity change, thus developing a hyperpolarization potential. The mean value of this second and opposite maximum was 639 mμ (*cf.* below).

Of certain interest is a constantly appearing dip in all the R-C spectral response curves in the course of the decay of the G curve towards the short wave end of the spectrum. This dip is most clearly seen in the bottom record of the R-G row of Fig. 1 (*cf.* also Figs. 5 and 6).

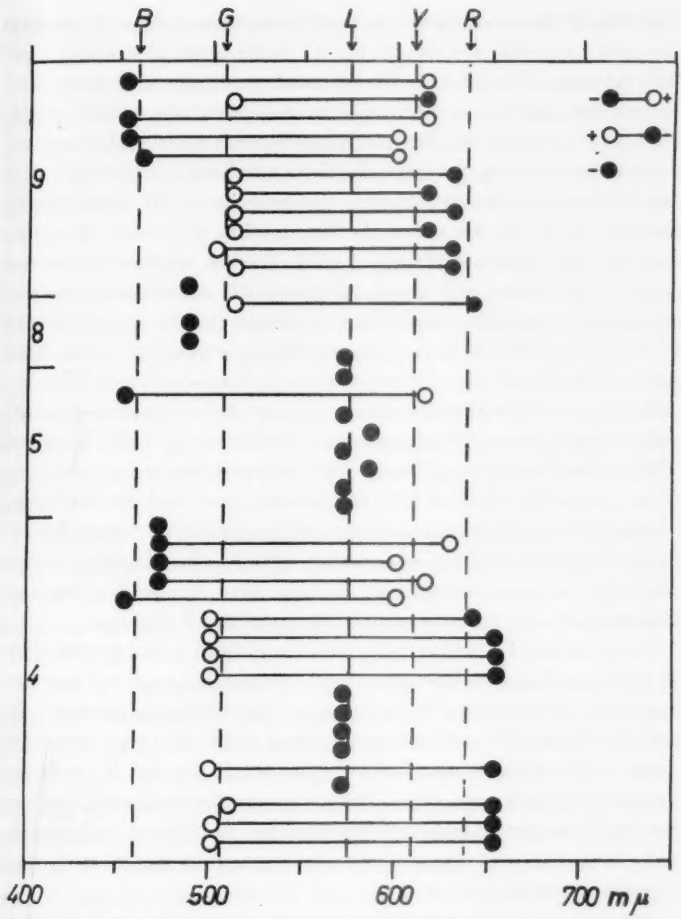

Fig. 3. Full description in text.

The Y-B spectral response curves (Fig. 1) showed a hyperpolarization potential with a mean maximum value at 460 mμ and a second and opposite depolarization maximum at a mean wavelength of 610 mμ. The maximal Y response amplitude was invariably found to be smaller and about one fifth of the maximal B response.

In Fig. 3 the wavelengths, at which maximum values of electric responses occurred, are shown for 41 consecutive observations on different cones. The filled circles represent hyperpolarizing potentials, whereas the open circles denote depolarizing potentials. A line joining two circles indicates that the two measurements were made consecutively without moving the electrode, the spectral response curve having two maxima of opposite polarity. The number of the experimental animal is shown to the left of the figure, while at the top the mean values of the five different maxima of the spectral response curves are indicated by a letter and associated arrow. All recordings showing a depolarization response maximum at G showed also an opposite one at R. The maximal R and G response amplitudes were of about the same size in all the recordings. However, of the 13 response curves (Fig. 3), with a hyperpolarization response maximum at B, four were obtained without a distinct associated maximum of opposite sign in the Y region.

The second and invariably smaller Y response maximum is not due to any unspecific effect of ERG components, since such are not detectable with the amplification used, the rods producing the b-wave having been completely removed (*Svaetichin* 1953 b). Interference by any other type of cones can also be excluded, since neither the response maximum nor the polarity agree with that of the Y response.

In this series of experiments both the amplification and the intensity of light stimulus were kept practically constant, the maximal response amplitudes in the spectral response curves presented being about 20—30 mV. The thresholds and maximal response amplitudes were about the same in the different types of receptor (L, R, G, and B) with the exception of the Y type, which displayed a smaller amplitude. Further, the fish cones showed no significant adaption, as reflected in the amplitude of the receptor response, when moderate light stimuli were used (*Svaetichin* 1953 a).

It is seen from Fig. 3 that there are two maxima grouped on either side of R at about 630 and 656 mμ. At present experimental data are insufficient to determine whether these two maxima really occur, or whether the appearance of two maxima instead of one is due to experimental errors. The spectral response curves, having their R maxima at about 656 mμ, were technically more successful recordings as compared to those with their maxima at about 630 mμ. Hence, the maximum at 656 mμ has been considered the more correct one, and this

value has also been used for the spectral response curve diagram in Fig. 9.

Concerning the distribution of the different types of receptor responses, the L type of spectral response curve predominated in recordings from cones in the superior half of the retina and in ora serrata. In the other parts, however, the R-G and Y-B types of responses were more common. Histological studies show that the single type of cone is generally the one prevalent in ora serrata, whereas in the central regions of the retina the twin cones predominate (*Friis* 1879, *Eigenmann* & *Shafer* 1900). Further, the cone action potentials recorded from the bream retina, which possesses only single cones (*Wunder* 1925), were invariably hyperpolarization potentials (*Svaetichin* 1953 a), *i.e.* possibly corresponding to the L type of cone. In this connection it has to be pointed out that in my previous studies (1953 a) the bream retina was solely used, with the exception of the experiments on the spectral response curves which were made on a mixed bream and perch material.

Consequently, it is a plausible conclusion that the L type of monophasic hyperpolarization responses were obtained from single cones, whereas the R-G and Y-B types of spectral response curves, each displaying two opposite maxima, represent recordings from twin cones (Figs. 1, 7, and 9).

As seen in the recordings in Figs. 1 and 4, a neutral point (np) appeared where the opposing components of the electrical responses cancel each other. At the bottom of Fig. 4 the single sweep tracings (a), (b) and (c) illustrate the shape of some individual cone action potentials elicited by different spectral light stimuli in recordings from cones producing an R-G type of spectral response curve. The (a) response is elicited by spectral light close to the R maximum of the spectral response curve, the (c) response is from a recording at the neutral point (np) between two opposite R and G maxima, and the (b) response represents a recording in the vicinity of this neutral point on the R side. These single sweep tracings are not recordings obtained simultaneously with the R-G spectral response curves presented above in Fig. 4; the letters (a), (b) and (c) on the spectral response curves (Fig. 4) merely indicate corresponding and similar responses on these curves.

When colorless light was used for stimulation of the R-G type of

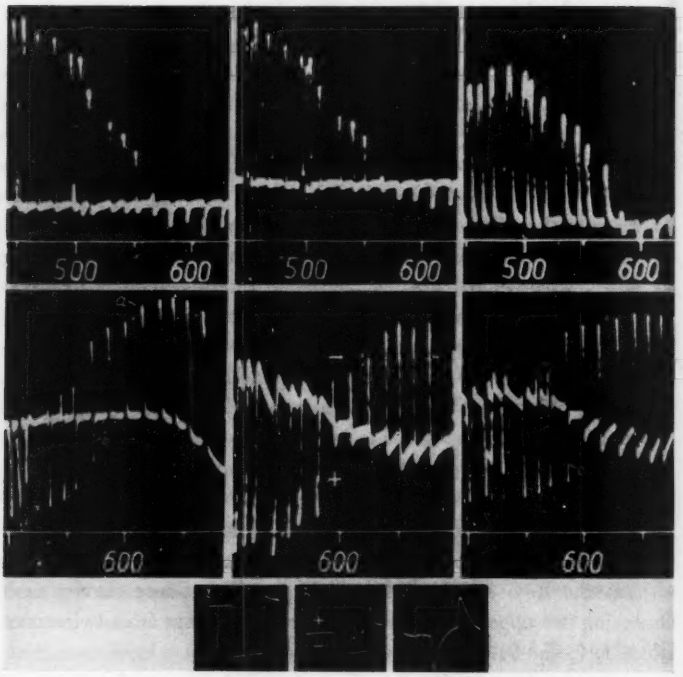

Fig. 4. Records in the region of neutral points of Y - B and R - G spectral response curves.

receptor, the response was a transient hyperpolarization potential appearing at "on" of the stimulus followed by a similar response of opposite polarity at "off" of the stimulus; actually a response which is identical with record (c) at the bottom of Fig. 4.

The reason why the R - G receptor, when stimulated by colorless light, or by spectral light close to the neutral point (np), produces only transient responses at "on" and "off", is that the latency (Figs. 1, 4, and 5) of the R response is less than that of the G response, and that the opposite R and G potentials, being of equal amplitudes, cancel each other except at "on" and at "off".

Unfortunately, the actual response of the Y - B receptor to a colorless light stimulus has not yet been studied in detail. Since, at least under

the experimental conditions described, considerable amplitude differences existed between the Y and B amplitudes at their spectral response maxima, the opposite Y and B potentials evoked by a colorless light stimulus do not seem to cancel each other completely.

The latency of the B response is also less than that of the Y response, and when testing with a wavelength of about 572 mμ, the response of the Y - B receptor is a pure "on-off" response (Figs. 1 and 4) similar to the response (c) in Fig. 4. However, the response amplitudes close to the neutral point of the Y - B spectral response curve are low as compared to the corresponding "on-off" responses of the R - G receptor. The neutral points of the R - G and Y - B spectral response curves are on the basis of the presented records situated at about 580 and 572 mμ respectively; points which approximately coincide with the maximum (574 mμ) of the spectral response curve of the L cone.

The outlines of typical recordings of R - G and Y - B spectral response curves are drawn in Fig. 5 a and b. The filled circles denote the amplitudes of the individual responses evoked by the spectral stimuli.

The neutral point (np), the point where a pure "on-off" response without a joining D.C. component was obtained, is indicated by the open circle on the zero line of the R - G spectral response curve (Fig. 5). To the left of the neutral point the D.C. component of each individual response is a hyperpolarization potential, and to the right of this point the D.C. component is a depolarization potential.

The continuous line through the neutral point shows approximately the level of the D.C. component of the responses.

The black dots in the neighbourhood of the neutral region connected by the dotted vertical lines correspond to the amplitudes of the transitory "on-off" deflections which are superimposed upon the D.C. component of each response.

The approximate configuration of some of the individual responses to spectral light stimuli has been drawn in the same diagram (Fig. 5 a), the associated arrow indicating the location of the corresponding response on the spectral response curve.

In the R - G spectral response curve the neutral point was situated about midway between the opposite R and G maxima, whereas in the Y - B curve the neutral point was closer to the Y maximum. It appeared that only in the case of the opposite maxima being of equal amplitudes, the distances between the neutral point and the maxima were also

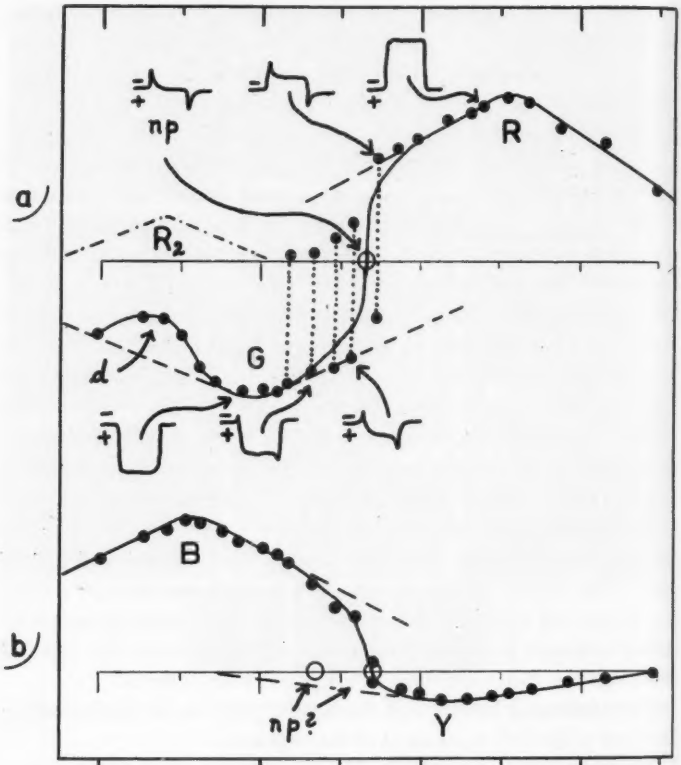

Fig. 5. Outlines of R - G and Y - B spectral response curves. Full description in text.

equal. For instance, in the curve in Fig. 5 a the maximal R response was somewhat higher than the G response resulting in a minute shift of the neutral point towards the G maximum. Similarly, the distances between the neutral point and the Y and B maxima were directly related to the amplitude differences between the Y and the B maxima.

The R and G maxima were generally equally high, the neutral point being situated at about 580 mμ. Further, under these recording con-

ditions the ratio between the amplitudes of the maximal Y and B responses appeared to be rather constant and about 1: 5.

The reason is not clear why the Y maximum of the Y - B spectral response curve showed an amplitude of only about one fifth of the amplitude of the B, R, G, and L maxima. Either the minor amplitude of the Y response is caused by some particular recording conditions, certain morphological features of the cones, or else the Y response is actually normally lower than the B response. However, the minor height of the Y maximum of the Y - B spectral response curve may be due to some particular recording conditions, and possibly the opposite Y and B receptor potentials are really equally high. In the case of the opposite maxima of the Y - B spectral response curve being of equal amplitudes, the neutral point of that curve would apparently also be situated midway between the opposite Y and B maxima, and further a pure "on-off" response would be evoked by a colorless light stimulus in harmony with the findings on the R - G receptor.

Assuming that the R, G, B, and Y response curves are approximately symmetrical on both sides of their maxima, the broken lines in Fig. 5 a and b have been drawn giving the course the curves would have taken without the mutual interaction in the parts where the curves overlap. In the neutral region, where the filled circles joined by vertical dotted lines indicate the maxima of the "on-off" responses, the curves show not a smooth but rather an unexpected sudden decay. Actually, close to the neutral point it is not a simple subtraction of opposite potentials but rather that the opposing receptor potentials appear to have a mutually inhibitory effect. This fact shows that the opponent receptor potentials are functionally interconnected and produced by a biological unit; — which apparently is the twin cones.

The B, G, and R spectral response curves drawn on a large scale in the diagram in Fig. 6 are based on the same recordings as the curves in Fig. 5. The response amplitudes (B=crosses, G=open circles, R=filled circles) are given in per cent of the maximal one obtained in each curve.

With the exception of the above mentioned dip (d) of the G curve and the distortion effect due to the overlapping, the spectral response curves are apparently identical in shape, showing an almost linear decay.

From the course of the response curves it appears that the dip of

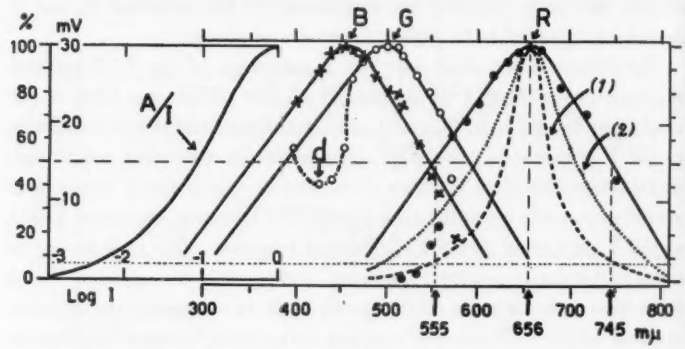

Fig. 6. Continuous lines indicate approximate course of B, G and R spectral response curves. Dotted (1) and broken (2) lines indicate spectral sensitivity curves computed by the aid of the A/I relation curve seen to the left of the diagram.

the G spectral response curve (d and associated arrow in Figs. 5 and 6) cannot be due to a submaximum of the G curve but rather to a submaximum at about 440 mμ of the R curve causing this reduction of the amplitude of the G curve at (d).

The presented spectral response curves have been obtained by varying the wavelength, the energy of the light stimulus being kept constant. The configuration of the spectral response curve would not be identical with a spectral sensitivity curve obtained on the same cone but their maxima would coincide. In determining the spectral sensitivity curve the reciprocal of light energy (1/I) needed for eliciting cone responses of equal amplitudes is plotted against the wavelength of the light stimulus used. Thus, in the latter case we are altering both the wavelength and the energy of the light stimulus, the response amplitude being kept constant. Since a change of the light energy occurs, we have to take into account the response amplitude—Log light intensity relation curve (the A/I curve). Such a curve obtained on a L type of cone is drawn to the left in Fig. 6. The curve is drawn on the basis of Fig. 6 in *Svaetichin* 1953 a, and Fig. 2 B in *Svaetichin & Jonasson* 1956. By aid of the A/I curve it is possible to transform a spectral response curve to a spectral sensitivity curve.

When determining the spectral sensitivity (luminosity) curve for the

human eye, the threshold value for perception of spectral lights of different wavelengths is used as index; working with retinal ganglion cells a certain impulse frequency is taken as index, and concerning the ERG a constant size of *e.g.* the b-wave can be used as index. In the case of determining the spectral sensitivity curve for a single cone, a certain constant amplitude of the cone action potential is used as index.

Choosing as index a cone response of a size corresponding to 100 per cent amplitude of the A/I curve seen in Fig. 6 in order to transform the R spectral response curve, we get an R spectral sensitivity curve denoted by number (1) in Fig. 6 (broken line).

The correctness of this computed spectral sensitivity curve (1) in Fig. 6 can be checked in the way described below. Using a light stimulus of the wavelength 565 mμ corresponding to the maximum of the R spectral response curve, we get at a certain intensity (I) of the light stimulus a response equal in size to the chosen 100 per cent amplitude index of the A/I curve. If on the other hand we are testing with the spectral lights of the wavelengths 555 and 745 mμ of the same intensity (I), a response is evoked of an amplitude of 50 per cent of the one at 565 mμ (see Fig. 6). From the A/I curve it appears that we have to increase the intensity of the light stimulus of the wavelengths 555 and 745 mμ by about 10 times (10$\times$I) in order to keep the chosen amplitude index constant at 100 per cent. According to the given definition of a spectral sensitivity curve the reciprocal value of the light intensity (I/10) is the one plotted against the wavelength. Consequently, the spectral sensitivity curve obtained with this actual index reaches at the wavelengths of 555 and 745 mμ a height of about 10 per cent of the maximal one at 656 mμ. In a similar way other points on the spectral sensitivity curve can be checked.

In case we are choosing a constant response index value corresponding to 50 per cent amplitude of the A/I curve, a broad curve for the spectral sensitivity is obtained (2, dotted line in Fig. 6) showing a course running intermediately between the spectral sensitivity curve (1) and the R spectral response curve. The closer the index is to the threshold for the excitation of the retinal neurons, the broader is the spectral sensitivity curve finally approaching the spectral response curve.

In this connection it is of interest to mention that from flicker fusion studies to conclude (*Svaetichin* 1956 b), the threshold for the excita-

tion of the retinal neurons appears to be at about 6 per cent of the maximal receptor response, corresponding to a cone action potential of about 2 mV amplitude. The horizontal line (dashes and dots) drawn at bottom of Fig. 6 indicates this threshold value.

As soon as the energy of the light stimulus is altered, the A/I curve is involved, causing the sigmoid decay of the spectral sensitivity curve. When too high an intensity of light stimulus is used, evoking the highest possible receptor response, the spectral response curve and the spectral sensitivity curve as well also tend to be broad. For instance, in the spectral response curve seen second from bottom in the R-G row in Fig. 1, the intensity of the light stimulus has been slightly too high, causing a broad curve particularly pronounced in the R region.

DISCUSSION

All the spectral response curves presented above were obtained with the microelectrode tip inserted into one cone only, since it is difficult to see how an electrode tip less than 0.1 μ in diameter could be inside two cones simultaneously, other cells than cones being excluded. The responses are to be considered membrane action potentials recorded across the plasma membrane of a cone (apparently the cone myoid or ellipsoid *Svaetichin* 1953 a). Consequently we have to accept: 1) in the L type of cone a hyperpolarization response of the cell membrane, 2) in the R-G and Y-B types of cones either a hyperpolarization or a depolarization response depending on the wavelength of the light stimulus used. In records from the bream retina (*Svaetichin* 1953 a), which has been shown to contain only single cones (*Wunder* 1925), the cone response to a light stimulus was invariably a hyperpolarization potential. However, the hyperpolarization action potential recorded across the photoreceptor plasma membrane was accompanied by impulse discharges of the retinal neurons in the layer of the horizontal and bipolar cells, continuing as long as the light stimulus lasted (Fig. 4 A in *Svaetichin* 1953 a);—a somewhat unexpected finding which is not compatible with present neurophysiological views.

The L type of spectral response curve with one chief maximum showing the same polarity throughout the spectrum is, of course, the type of response one would expect to elicit from a single cone. The additional existence of several submaxima is reasonably explained by

the presence of more than one photopigment in the outer segment of this cone, which has tentatively been indicated by the straight continuous lines in Fig. 2. It appeared (see Results) that the distribution of the L type of recordings in the retina agrees well with the histologically known distribution of the single type of cone in the fish retina. In the bream retina, in which only the single type of cone occurs, the recordings were invariably hyperpolarization potentials (*Svaetichin* 1953 a) *i.e.* apparently corresponding to the L type of response. All these findings support the view that the L type of response was actually recorded from the single type of cone.

As far as the R-G and Y-B types of spectral response curves are concerned, we still have to accept the fact that they have been recorded with the electrode inserted into one cone only. In this event it is, however, hard to find a plausible explanation of the finding that the action potential undergoes polarity changes depending on the wavelength of the spectral stimulus used, and besides that the opponent responses appear to display a mutually inhibitory effect. In the R-G type of spectral response curve the opponent potentials are of about the same amplitude at their maxima, whereas in the Y-B type there is a constant amplitude difference between the maxima. Moreover, the R-G type reacts to a colorless stimulus with an "on-off" response, the opposing receptor potentials cancelling each other everywhere except at "on" and at "off". As mentioned (see Results), this is due to a latency difference between the two opponent processes. Primarily two independent photochemical processes with different latencies might occur in the cone outer segment, the R and G responses recorded across the plasma membrane of the cone ellipsoid or myoid being their electric consequences.

It is plausible that the two suggested primary photochemical processes could occur in one and the same outer segment. Actually, as suggested above, there even appears to be several different photopigments in the same L cone outer segment. In this case, however, the electric response showed invariably the same polarity. Hence, there are reasons to seek another explanation for the subtraction of opponent potentials and the mutual counteraction effect seen in the R-G and Y-B types of spectral response curve recordings.

As mentioned (see Results) there is a certain correlation between the histological distribution of the twin cones and the single cones and

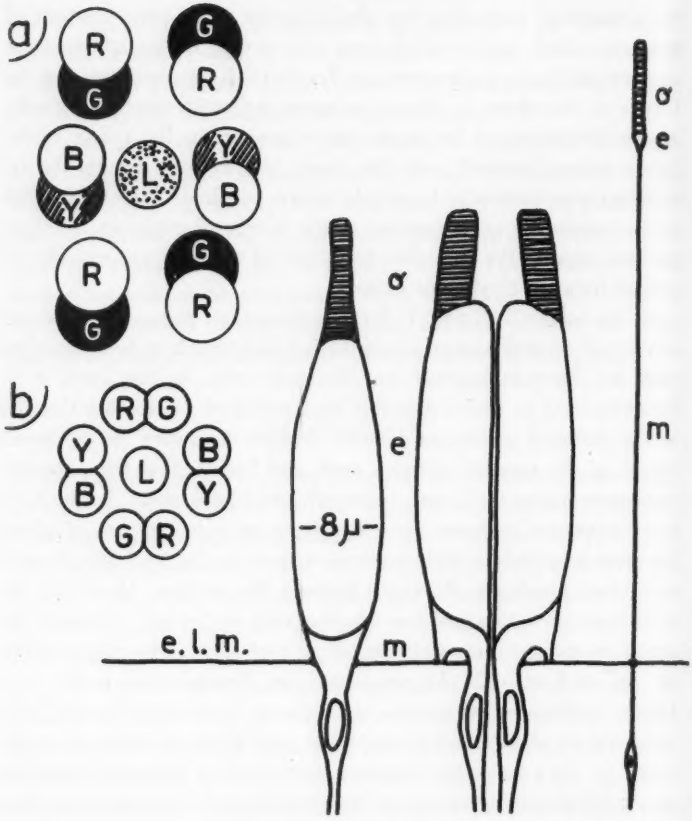

Fig. 7. Single and twin cones with a rod to the right of the cones in the *Teleost* retina, redrawn from *Walls* 1942. Cone pattern in the retina of some *Gecko* genera (a) schematically redrawn from *Underwood* 1951. Commonest cone pattern in fish (b) redrawn from *Eigenmann & Shafer* 1900. Suggested functional signification of different cone types tentatively indicated by letters. Labelling: (o) outer segment, (e) ellipsoid, (m) myoid and (e.l.m.) external limiting membrane.

the distribution of the different types of recordings obtained from the retina, offering support for the view that the R - G and Y - B types of responses were elicited from the twin cones.

Histological observations indicate that the connection between the twin cones is very close; indeed, they seem actually to be fused together (Fig. 7). Thus it is reasonable to suggest that the large contact surface (300 μ^2) formed by the plasma membranes between the cones, serves as a giant synapse. This suggestion is in good agreement with electron microscopic findings (*Sjöstrand* & *Elfvin*, personal communication 1956) proving the existence of two closely spaced plasma membranes at the border between the twin cones; a structure similar to that found in retinal synaptic regions (*Sjöstrand* 1954, 1955).

Another possibility would be that the closely spaced plasma membranes separating the members of the twin cone are electrically non-polarized, and that in this case the twin cone could be considered to be one cell in electrophysiological respects.

Anyhow, in the twin cone there exists morphologically an extraordinary close connection between two receptor cells (Fig. 7) offering the structural element, on the basis of which a close functional interaction between two cones could occur. It is reasonable to suggest that the twin cones produce potentials of opposite signs, one of the twin cone plasma membranes (G & Y) being depolarized and the other (R & B) hyperpolarized when adequately stimulated; the potential of the gaining polarity being impressed upon the cell membrane of the other cone (*cf.* the suggestions by *Müller* 1897, pp. 9, 21, 25). On these lines it could be explained, for instance why the R-G and Y-B spectral response curves show the same amplitude relation between their maxima irrespective of from which member of the twin cone the actual recording has been made. The observed subtraction of opponent potentials and the mutual inhibition would also be interpreted on the basis of these suggestions.

Concerning the significance of the different receptors classified on the basis of the typical spectral response curves obtained, it appears that the L type of cone is well adapted to master the photopic luminosity mechanism, since it reacts to all qualitatively different light stimuli with the same unspecific response, whereas the responses of the R-G and Y-B types of cones are differentiated in a way that renders it reasonable to assume that they are responsible for the color vision mechanism.

Assuming the L type of cone delivers the signal for the photopic luminosity in the fish retina, the maximum of the spectral response

curve of the L cone would coincide with the crest value of a photopic luminosity curve obtained on fish. By aid of an optomotor response, both scotopic and photopic luminosity curves have been determined on the sunfish *(Lepomis)* by *Grundfest* (1931, 1932).

The outlines of two typical spectral response curves of the L type have been drawn in Fig. 8 (dotted and broken lines), the response amplitudes (ordinates) given in percent of the maximal ones obtained (572 and 586 mμ), have been plotted against wavelengths (abscisse) in mμ. In the same diagram two of *Grundfest*'s photopic spectral sensitivity curves have been redrawn (continuous lines), representing the two extreme limits of the scattering of the maxima of the curves obtained at high intensities of spectral illumination.

The limits for the scattering of the maxima of *Grundfest*'s photopic luminosity curves are about 560—600 mμ, and the corresponding values for the L type of spectral response curves are 572—586 mμ (see values on top of Fig. 8).

The mean maxima of the B, G, Y, and R types of spectral response curves have been indicated by letters and associated arrows at bottom of Fig. 8. Evidently, only the L type of spectral response curve agrees perfectly with the photopic luminosity curve of the sunfish, the maximum of the Y type of spectral response curve also being rather close. The minor scattering of the maxima obtained on single cone recordings is reasonable. Further, the photopic luminosity curve of the sunfish and the spectral response curve of the L type of cone show to some extent a similar course, the decay being less steep towards the short wave end of the spectrum.

It was shown that the amplitudes of the receptor potentials evoked by equal energy light stimuli, were about equal at the maxima of all the different types of spectral response curves obtained with the exception of the Y curve. Further, the threshold values of the different types of cones were also about the same.

In case another type of cone in addition to the L type should contribute to the photopic luminosity mechanism, the crest of the fish photopic luminosity curve would coincide also with the maxima of the other types of spectral response curves. This is evidently not true, since the photopic luminosity curve of the fish coincides exclusively with the L type of the spectral response curve. Consequently: a) the L type of cone (morphologically the single type of cone, see Fig. 7) delivers the

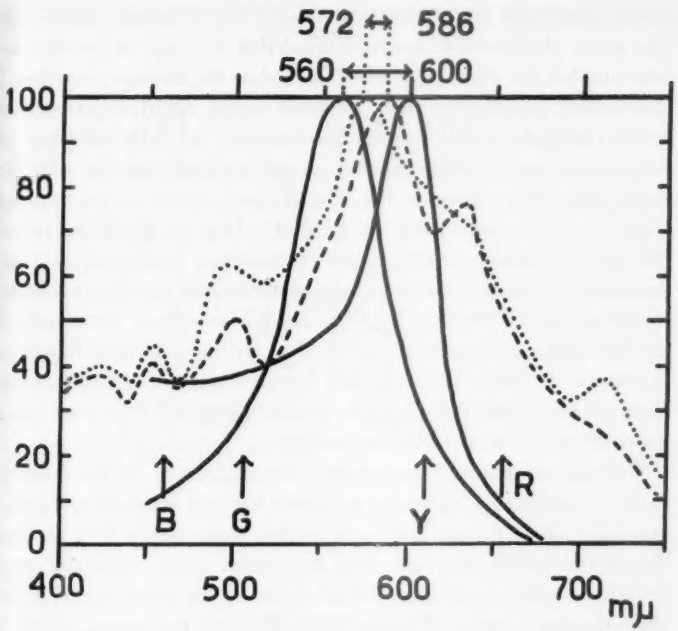

Fig. 8. Photopic luminosity curves (continuous lines) obtained on sunfish using the optomotor response, redrawn from *Grundfest* 1932. L types of spectral response curves obtained from single fish cones, indicated by broken and dotted lines. Limits of scattering for maxima given on top of figure. The maxima of the B, G, Y and R spectral response curves obtained on fish cones denoted at bottom of figure by letters and associated arrows.

signal for the photopic luminosity in fish; b) the R-G and Y-B types of cone do not contribute to the luminosity mechanism, and c) without doubt, the R-G and Y-B types of cone (the twin cones in the retina) constitute the chromoreceptors in fish.

In a chapter of his recent book, *Prince* (1956) describes the double and twin cones which so regularly appear in diurnal vertebrates. However, there is no reasonable explanation offered for their possible function. *Underwood* (1951) who particularly examined the reptilian retina, describes the cone pattern in the retina of some *Gecko* genera. He writes: "As has been described for other *Geckos*, the single and

double visual cells are in horizontal rows, a row of singles alternating with a row of doubles. It has been noted that in a row of doubles the chief member lies alternately above and below the accessory member." (The latter, possibly in order to avoid undue electrical interaction between adjacent double cones!) He describes two different types of double cones which are arranged in a regular pattern together with the single cones. The scheme in Fig. 7 a has been drawn on the basis of Fig. 2 in *Underwood*'s publication (1951). Thus, in the *Gecko* retina five morphologically different types of cones have been described, and the suggested functional significance of these cones has been indicated tentatively by the letters in Fig. 7 a. The corresponding visual unit of the fish retina is illustrated in Fig. 7 b (redrawn from a figure in *Eigenmann* & *Shafer* 1900). In fish, however, morphologic differences have not been observed. Electron microscopy of the twin cone outer segments would be of particular interest.

With the exception of the spectral response curves, in the determination of which a mixed bream and perch material was used, my previous study (1953 a) was made only on the bream retina. It is obvious that the responses recorded from the bream cones constantly were hyperpolarization potentials. Actually, the bream retina contains exclusively single cones (*Wunder* 1925). Possibly the bream, which is living close to the bottom in the darkness, is color blind, possessing only the L type of single cone. It would be of interest to know if color blind animals in general possess only the L type of cone (cone monochromats!).

The crest of the photopic luminosity curve of the human eye (555 mμ) is shifted about 20 mμ towards the short wave end of the spectrum as compared to the maximum of the L type of spectral response curves of fish (Figs. 1, 2, and 9). In this connection it is of interest to mention that a corresponding shift exists between the absorption maxima of rhodopsin and porphyrsopsin, the scotopic photopigments of human and fish respectively.

The spectral response curves R - G and Y - B (Figs. 1, 9) bear a strong resemblance to the processes suggested by *Hering* (1878, 1925, 1931) in his opponent color theory. The counteraction effect of the opponent receptor potentials and the existence of a neutral point between two opponent receptor response maxima is compatible with the mutual exclusion effect of opposing color pairs as stated by *Hering*.

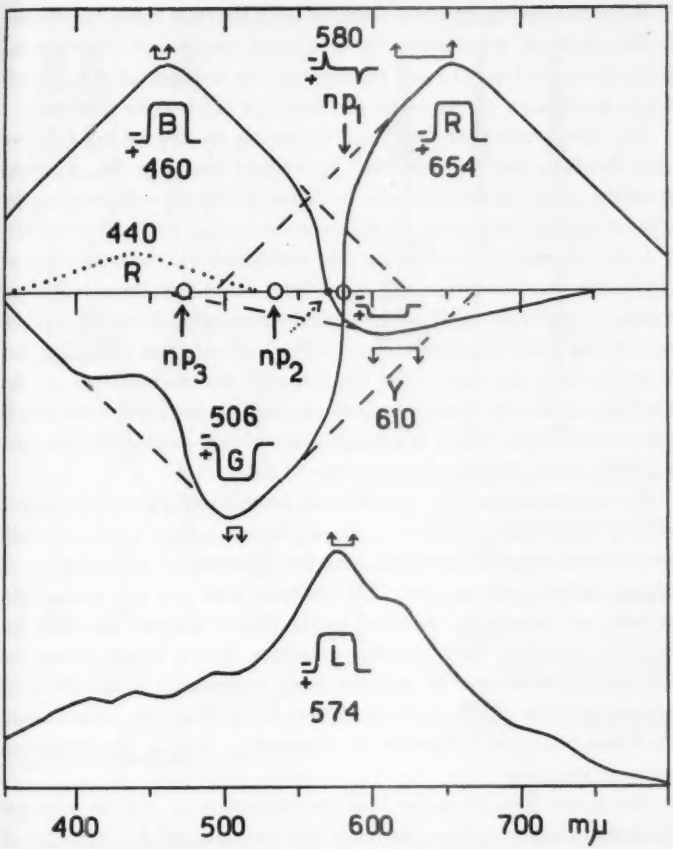

Fig. 9. Scheme of spectral response curves obtained from fish cones. Arrows on top of the crest of each curve give scattering of maxima. Neutral points denoted by open circles on zero line. The neutral points of R - G, Y - B and R_2 - G are given by np_1, np_2, and np_3 respectively. The location of np_2, and the existence of np_3 is uncertain.

The diagrammatic picture in Fig. 9 is in good conformity with the *Hering* theory. The maxima for the schematic spectral response curves are the mean values from Fig. 3, except the R maximum for which 656 mμ has been taken (for the reason see Results). The R - G and

Y-B spectral response curves obtained from the twin cones correspond to the opponent color pairs, the L spectral response curve recorded from the single type of cone, representing the separate photopic luminosity mechanism corresponding to *Hering*'s black-white substance.

The submaximum of the R curve, causing the dip of the G curve (see Results), has been denoted R_2 (dotted line, Fig. 9). A corresponding subcomponent will also be found in diagrams illustrating the *Hering* theory (see *e.g. v. Tschermak—Seysenegg* 1929, 1947, 1952).

It is now generally recognized that additionally to the main crest of the human photopic luminosity curve, there are at least three additional humps (*e.g. Wright* 1952) which possibly correspond to the submaxima seen on the spectral response curve of the L cone in fish (Fig. 2). This is most likely the case, since these humps are also present on the photopic luminosity curve obtained on totally color blind cone monochromats (*Wright* 1952). It appears reasonable to assume that the cone monochromats possess only the L type of cone.

It is an astonishing fact that the cone reacts to a light stimulus either with a hyperpolarization or a depolarization action potential. Both polarities of receptor potentials must be accompanied by excitation of certain retinal neurons, since, for instance, both red and green light stimuli are necessarily signalled to the higher centers, although the receptor responses have opposite polarities. In the bream retina the hyperpolarization type of response solely represented is proved to be accompanied by impulse activity (*Svaetichin* 1953 a); the more reason, therefore, to expect excitation in connection with a depolarization receptor potential.

Hence, we have to accept that depolarization as well as hyperpolarization receptor action potentials are accompanied by excitation of retinal neurons. Cathodal excitation and anodal inhibition actually are basic conceptions in neurophysiology, and to say the opposite is nonsense. In terms of a strictly chemical synaptic transmission theory the polarity would be unimportant. However, the neuronal activity is constantly accompanied by potentials, and the polarity is certainly significant.

In this connection it should be mentioned that experiments carried out on dorsal root ganglion cells (described by *Svaetichin* at the Scandinavian Physiological Congress in Helsingfors, 1954, *Svaetichin* 1956 c) show that an anodal stimulus, when locally applied to a

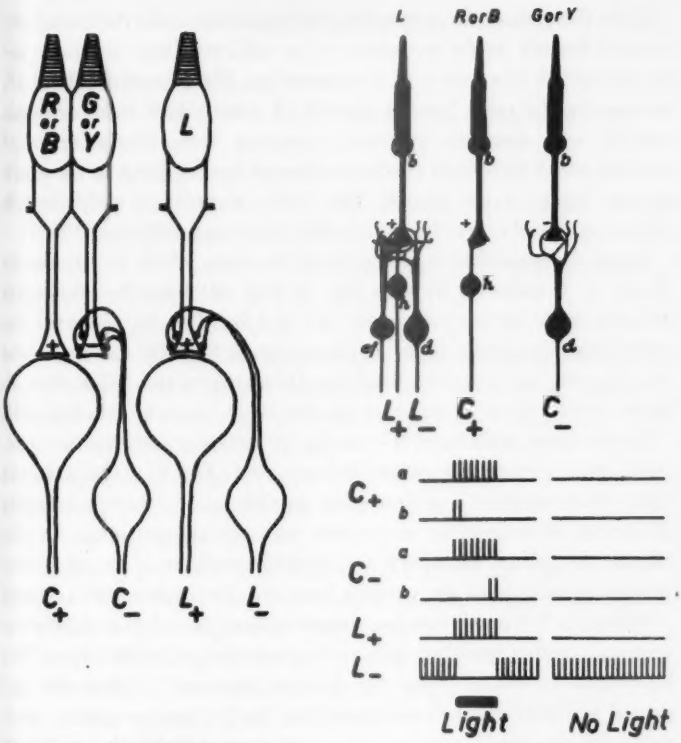

Fig. 10 See description in text.

perikaryon pole, opposite to the axon hillock, has a lower threshold than the cathodal one. The impulse recorded from the cell body was found to start at a lower threshold and at "on" of the stimulus, when an anodal stimulation was used, whereas the impulse started at "off" and showed a higher threshold when a cathodal stimulus was applied.

The reason for such an unexpected finding was shown to be that the excitation actually occurred (and the action potential started) in the initial segment of the axon. An anode on the perikaryon pole opposite to the axon hillock produces outward currents through the axonal membrane of the initial segment and is thus equal to a cathode applied to the juxta-axonal pole of the cell body or to the initial segment.

In the fish retina a large number of cone pedicles make their synaptic contacts directly on the perikarya of the adjacent large neurons (see the micrograph of a section of a bream retina, Fig. 1 *Svaetichin* 1953 a), whereas on the other hand a number of other cones make synaptic contacts with dendritic processes emerging from bipolar types of neurons, which have their perikarya situated further down in the inner nuclear layer of the retina. This is in accordance with electron microscopic studies by *Sjöstrand* (personal communication, 1956).

Hence, it is possible to assume that the cones which hyperpolarize (R, B, or L indicated by plus sign in Fig. 10) have their synaptic pedicles on the perikaryon proper (C+ and L−, Fig. 10), whereas the cones which depolarize (G or Y, minus sign in Fig. 10) make synaptic contacts with the axon-like dendrites belonging to the cell bodies situated further down in the inner nuclear layer (C− and L−, Fig. 10).

On the upper right hand side of Fig. 10 the same ideas are incorporated into a picture taken from *Polyak* (1941, Fig. 97). Below to the right of the diagram are shown the possible spike response patterns of the retinal neurons in accordance with the interpretation. In this scheme the mutual excitatory and inhibitory effects of the opponent receptor potentials on the neurons have also been taken into account.

The single L type of cone has tentatively been joined to two different neurons, possibly resulting alternatively in excitation or inhibition. The L+ neuron is excited when the L cone responds, whereas the L− neuron is inhibited. It is sugegsted that the L+ neuron signals luminosity, whereas the L− neuron, when continuously discharging impulses, signals "black", the signal for "black" being inhibited when the L cone delivers its receptor potential.

Considering *Granit*'s (1955, pp. 32, 140, 190) criticism of my earlier findings (1953 a), I am happy to see *Motokawa*'s confirmation (presented at the Brussels Congress 1956) of the hyperpolarization potential.

The spectral response curves described by *Motokawa* (1956), which consisted of one main crest and three additional humps, showed the following maxima: 470, 550, 600, and 650 mμ, values which are similar to those given in my earlier paper (1953 a), viz: 450, 550, 600, and 650 mμ. *Motokawa*'s results are in other respects to some extent diverging from the data in my present study and also from those of my earlier study. However, the discrepancies seem to be caused by differences between the fish species used.

REFERENCES

Bauer, V., Arch. ges. Physiol. 1910. *133.* 7.

Eigenmann, C. H. & G. D. Schafer, The American Naturalist. 1900. *34.* 109.

Friis, G., Fiskeøiet. Copenhagen 1897.

Frisch, K. v., Zool. Jb., Abt. allg. Zool. Physiol., 1913. *34.* 43

— Z. vergl. Physiol., 1925. *2.* 393.

Granit, R., & G. Svaetichin, Uppsala Läkaref. förh. 1939. *65.* 161.

Granit, R., Nature, 1943. *151.* 11.

— J. Neurophysiol. 1945. *8.* 195.

— Sensory Mechanisms of the Retina. Oxford Univ. Press. 1947.

— Receptors and Sensory Perception. Yale Univ. Press. 1955.

Grundfest, H., J. Gen. Physiol. 1931. *15.* 307.

— J. Gen. Physiol. 1932. *15.* 507.

Hamburger, V., Z. vergl. Physiol. 1926. *4.* 286.

Helmholtz, H. v., Ann. d. Physik. 1852. *87.* 45.

— Ann. d. Physik. 1853. *94.* 1.

— Hdb. d. physiol. Optik. Leipzig 1911 (First ed. 1866).

Hering, E., Zur Lehre vom Lichtsinn, Wien 1878 (First ed. 1876).

— Hdb. d. ges. Augenheilk. 1925. *3.* 12. 1.

Linksz, A., Physiology of the Eye. *2.* New York 1952.

Motokawa, K., J. Neurophysiol. 1949. *12.* 291.

— J. Neurophysiol. 1949. *12.* 475.

Motokawa, K., & K. Isobe, J. Optic. Soc. America. 1955. *45.* 79.

Motokawa, K., Abstracts of XXth International Physiological Congress. 1956. 6662.

Müller, G. E., Z. Psychol. u. Sinnesphysiol. 1896. *10.* 1.

— Z. Psychol. u. Sinnesphysiol. 1897. *14.* 1.

Polyak, S. L., The Retina. Chicago Univ. Press. 1941.

Prince, J. H., Compartive Anatomy of the Eye. Springfield 1956.

Sjöstrand, F. S., Z. wiss. Mikrosk. 1954. *62.* 65.

— Symp. 8th Congr. Cell Biol. International Union of Biological Sciences, Series B. 1955. *21.* 222.

— Personal communication. 1956.

Sjöstrand, F. S. & L. Elfvin, Personal communication. 1956.

Svaetichin, G., Acta Physiol. Scand. 1953. *29.* Suppl. 106. 565.

— Acta Physiol. Scand. 1953. *29.* Suppl. 106. 601.

Svaetichin, G., Acta Physiol. Scand. 1954. *31*. Suppl. 114. 52.
— Acta Physiol. Scand. 1956. This Supplement.
Svaetichin, G., & R. Jonasson, Acta Physiol. Scand. 1956. This Supplement.
Tschermak-Seysenegg, A. v., Hdb. d. norm. u. path. Physiol. 1929. *12/1*. 295. 833.
— Einführung in die physiologische Optik. Wien 1947.
— Introduction to Physiological Optics. Springfield 1952.
Underwood, G., Nature. 1951. *167*. 183.
Walls, G., The Vertebrate Eye and its Adaptive Radiations. Michigan 1942.
Wolff, H., Z. vergl. Physiol. 1925. *3*. 279.
Wright, W. D., J. Optic. Soc. America. 1952. *42*. 509.
Wunder, W., Z. vergl. Physiol. 1925. *3*. 1.
Young, T., Lectures in Natural Philosophy. 1807. *2*. 613.

III. RECEPTOR MECHANISMS FOR FLICKER AND FUSION

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Several authors are of the opinion that the mechanisms determining flicker and fusion are chiefly situated in the brain, whereas others suppose that the critical flicker fusion frequency primarily depends on retinal functional limitations. The influence different substances (*e.g.* alcohol, barbiturates) have on the critical fusion frequency, has been thought to be caused by effects on the central nervous system. Thus, the location of the structures responsible for flicker and fusion still seems to be uncertain. A complete review of the literature of the flicker fusion phenomena is given by *Landis* (1953, 1954).

The human ERG, evoked by intermittent light stimuli, showed a fusion of the electrical responses at a frequency rather close to the subjective maximal fusion frequency (*Dodt* 1951 a, b). From these findings it could be concluded that the mechanisms for flicker fusion are reflected in the ERG and would thus be situated in the retina. If we consider the ERG as a practically pure receptor potential (*Ottoson* & *Svaetichin* 1952, 1953, *Svaetichin* 1953 b, and *Brindley* 1956) the flicker fusion experiments done on the human ERG would strongly support the view that the mechanisms determining the critical flicker fusion frequency, are situated in the receptors. From experiments on single cones, *Svaetichin* (1953 a) concluded that the characteristics of flicker and fusion are primarily determined by time constants of the cone action potential.

Using a method based on the optomotor response, it was possible to obtain curves on living sunfish showing the relation between critical fusion frequency and Log intensity of light stimulus (*Wolf & Zerrahn-Wolf* 1936). In Fig. 1 taken from *Bartley* 1951, the curve to the left is obtained on the sunfish by use of the optomotor response method (*Wolf* & *Zerrahn-Wolf* 1936) and the curve to the right is a corresponding flicker fusion curve obtained on man (*Crozier, Wolf* & *Zerrahn-Wolf* 1937).

It is interesting to notice the striking resemblance (*Svaetichin* 1953 a) between the steep "cone part" of the flicker curve of sunfish (Fig. 1) and the curve showing the relation between the amplitude of the cone

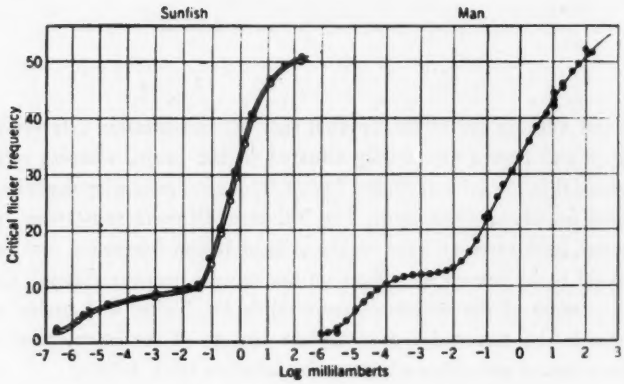

Fig. 1. Curves showing the relation between flicker fusion frequency and Log intensity of the light stimulus (taken from *Bartley* 1951). The curve to the left obtained on sunfish by use of the optomotor response method (*Wolf* & *Zerrahn-Wolf* 1936). To the right is a human flicker fusion curve (*Crozier, Wolf* & *Zerrahn-Wolf* 1937).

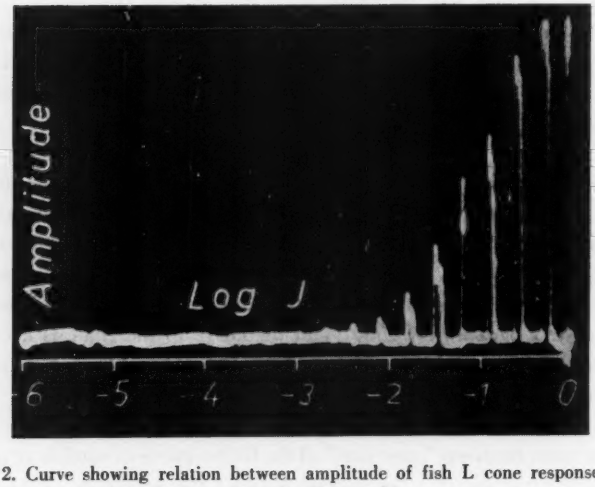

Fig. 2. Curve showing relation between amplitude of fish L cone response and Log intensity of light stimulus (part I, this suppl.).

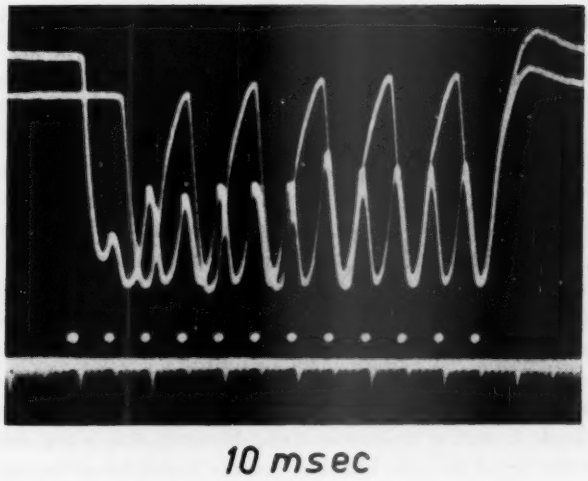

Fig. 3. Two photographically superimposed cone response curves evoked by intermittent stimuli of frequencies 10 c/s and 20 c/s, respectively. Flashes (duration 100 μ sec.) indicated upwards on time scale. Time 10 + 50 + 100 msec.

response of fish and the Log intensity of the light stimulus (Fig. 2 and part I, this suppl.). Both curves cover about three Log units.

The behavior of the cone action potential, when evoked by intermittent light stimuli, is described by *Svaetichin* in 1953 (1953 a, Figs. 14, 15), in which paper it was shown that the rise and decay times of the receptor responses determined the fusion of the cone action potentials at a certain frequency, this frequency being of the same order of magnitude as the maximal fusion frequency obtained on sunfish by the optomotor method. Two individual responses evoked subsequently by intermittent light stimuli of the frequencies 10 and 20 c/s, respectively, from the same L type of fish cone, have been photographically superimposed in Fig. 3. Electronic flashes of a duration of 100 μ sec. were used. It appeared that the amplitudes of the transitory cone responses (*i.e.* ripple on top) were reduced to about half, when the frequency of the flashes was doubled. This fact is explainable on a simple geometrical basis. If the transitory spike-like action potentials evoked by single flashes should have strictly angular shapes, each

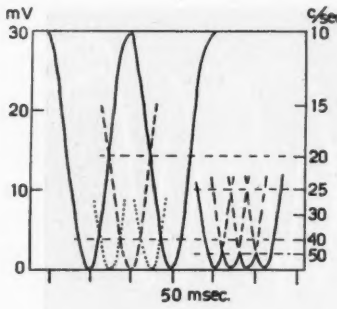

Fig. 4. Schematically drawn transitory cone potentials of maximal possible total amplitudes (30 mV), illustrating decrease of amplitude of ripple on top of cone responses, when frequency of flash stimuli is increased above 10 c/s.

doubling of the flash frequency would necessarily reduce the response amplitude to half of the previous one. However, this is not the case, since the tips of the spike-like responses are rounded and the course of the rise and decay is not straight. In Fig. 4 it is shown approximately how this deviation from the angular form influences the reduction of the amplitude of the cone responses (*i.e.* ripple on top), when the frequency of the flashes is increased. It is seen that the responses are reduced to less than half by further doubling of the frequency from 20 c/s upwards, fusion of the responses being completed close to 50 c/s.

The frequency of 10 c/s is about the frequency upwards from which the individual transitory spike-like potentials start to fuse (Fig. 3, *cf.* also Fig. 14, *Svaetichin* 1953 a). Further, as appears from Fig. 1 (sunfish), 10 c/s is also the frequency where the steep "cone part" of the flicker fusion curve commences.

Assuming that the 10 c/s receptor potentials (the spike-like responses), seen in Fig. 3, possess a critical threshold amplitude high enough to evoke rhytmical neuronal spikes in pace with the receptor potentials, a doubling of the stimulating flash frequency would result in a reduction to about half of the amplitude of the receptor potentials, and thus the amplitude of the receptor potentials would be below the critical threshold. In order to again reach the critical threshold amplitude for initiating of impulses in the bipolar cells, the intensity of the light stimuli has to be increased. The degree by which the light intensity has to be increased, is determined by the response amplitude — Log light intensity curve shown in Fig. 2 (!), and consequently the sigmoid

shape of this curve is necessarily reflected in the flicker fusion curve seen in Fig. 1 (sunfish). Each time the frequency of the intermittent stimuli has been doubled, resulting in a decrease to about half of the amplitude of the receptor responses, one has to increase the light intensity of the flashes by about ten times (Fig. 2) in order to keep the receptor response amplitude constant when the frequency of the light stimuli is increased.

The amplitude of the cone action potential was maximally about 30 mV, when evoked by a continuous light stimulus or intermittent light stimuli of a maximal frequency of about 10 c/s. As appears from Fig. 4, the amplitude of the spike responses was only about 6 % (2 mV) of the maximal one, when the flash frequency was close to 50 c/s, which is the maximal frequency for flicker fusion obtained on the sunfish. From these experiments it can be concluded that the critical threshold amplitude of the receptor potentials is about 2 mV for excitation of the retinal bipolar cells.

It can further be concluded that the flicker fusion curve obtained on man (Fig. 1) reflects the response amplitude — Log light intensity relation curve of human cones. Apparently, this curve covers four Log units in man. The rise and decay times of the responses of the human cones seem to be shorter (higher body temperature) than those of the fish cones, since the steep "cone part" of the human flicker fusion curve commences at a higher frequency (15 c/s), the maximal fusion frequency in man being higher than that of the fish.

It is well known that alcohol lowers the flicker fusion frequency in man (*e.g. Goldberg* 1943, *cf.* also ERG experiments by *Bernhard & Skoglund* 1941). A drop of diluted ethyl-alcohol instilled into the fish retina lengthened the rise and decay times of the cone action potentials. Apparently, different substances influence the time constants of the receptor potentials, resulting in changes in the flicker fusion frequency.

On the basis of the experiments on single cones of fish, it can be concluded that the main characteristics of flicker and fusion are determined by the time constants of the photoreceptor responses.

It cannot be excluded, however, that certain subtle phenomena noticed in the analysis of the perception of flicker and fusion, may be caused by an activity at different levels of the visual pathways (*Svaetichin* 1953 a).

REFERENCES

Bartley, S. H., Hdb. exp. Psychol., ed. Stevens. New York 1951. 921.

Bernhard, C. G., & *R. Skoglund*, Acta Physiol. Scand. 1941. *2*. 10.

Brindley, G. S., J. Physiol. 1956. *28*. 360.

Crozier, W. J., *E. Wolf*, & *G. Zerrahn-Wolf*, J. Gen. Physiol. 1937. *21*. 203.

Dodt, E., Nature. 1951. *168*. 738.

Goldberg, L., Acta Physiol. Scand. 1943. *5*. 1.

Landis, C., An Annotated Bibliography of Flicker Fusion Phenomena. Ann Arbor Mich. 1953.

— Physiol. Rev. 1954. *34*. 256.

Ottoson, D. & *G. Svaetichin*, Acta Physiol. Scand. 1953. *29*. Suppl. 106. 538.

Svaetichin, G., Acta Physiol. Scand. 1953. *29*. Suppl. 106. 565.

Wolf, E. & *G. Zerrahn-Wolf*, J. Gen. Physiol. 1936. *19*. 495.

IV. NOTES ON THE ERG ANALYSIS

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Some mistakes have first to be admitted. In the suggested component analysis of the ERG by *Ottoson & Svaetichin* (1952), the assumed polarity of the "off" response of the rods was positive and the polarity of that of the cones was negative. However, the "off" responses proved to be of a polarity opposite to this, which is in agreement with the interpretation given by *Svaetichin* (1953 b). On the basis of my present experiences I am inclined to believe that the differences between the local ERG responses recorded from central and peripheral parts of the frog retina, as reported by *Ottoson & Svaetichin* (1952), could possibly have been caused to some extent by local malfunction of the retina close to the recording electrode (*cf. Brindley* 1956).

Origin of the ERG.

Concerning the intraretinal recording of slow potential changes, *Tomita & Torihara* (1956) and *Brindley* (1956) have proved the existence of slow potentials in the neuronal layers of the retina. However, these potentials do not contribute in any significant way to the ERG recorded in the conventional manner with electrodes across the eye bulb or the retina (*Brindley* 1956). These "*slow neuroretinal potentials*" seem most likely to be comparable with the slow potentials of the spinal cord, and in analogy with the spinal root potentials the optic nerve potentials apparently reflect these slow neuroretinal potentials. The ERG, which is generated by the basal parts of the rods and cones (*Ottoson & Svaetichin* 1953, and part I, this suppl.), is of such a predominating amplitude, as compared to the slow neuroretinal potentials, that the latter has no chance to contribute significantly to the ERG. The closely packed photoreceptors, kept together by the external limiting membrane, produce radially oriented potential fields when stimulated by light (*cf. Brindley* 1956).

From the papers by *Tomita & Torihara* (1956, p. 130) it is evident that they too realize that the slow neuroretinal potentials (focal potentials) do not significantly contribute to the ERG, since they write: "As mentioned elsewhere (24), the focal potentials are very susceptible

to aging at higher temperatures. At 17° C. and above, *they usually disappear in 10 minutes or less, while the ERG does not change much.*" However, in the next paragraph they say, in contradiction to this: "The focal potentials are most distinct at the bipolar layer which generates the main part of the ERG."

From the experiments by *Ottoson & Svaetichin* (1953 Fig. 10) we know that the slow potentials recorded at "on" and "off" from the optic nerve are much more thermosensitive than the ERG itself. This finding is in agreement with the interpretation given above that the slow neuroretinal potentials described by *Tomita* and collaborators are closely interconnected with the slow potentials of the optic nerve. *Bernhard* (1942) showed that the b-wave of the ERG appears much earlier than the optic nerve response, and it has been shown by *Schubert* (1953) that the ERG and the optic nerve responses reflect different processes.

The discrepancies between the findings of *Tomita* and collaborators (1950, 1952) and *Ottoson & Svaetichin* (1952, 1953) now seem to have been clarified, thanks to the work of *Tomita & Torihara* (1956) and *Brindley* (1956). Both groups of investigators were both right and wrong, since there obviously do exist slow neuroretinal potentials, but on the other hand it is also true that these potentials do not significantly contribute to the ERG.

Already *Kühne & Steiner* (1880, 1881) observed that the optic nerve response is easily abolished, being sensitive to aging, maltreatment, etc., whereas the configuration of all the components of the ERG remains unchanged for hours. Since *Ottoson & Svaetichin* (1952, 1953), in their microelectrode experiments, were primarily interested in determining the origin of the ERG, they did not concern themselves with the response of the optic nerve, which response is apparently dependent on the persistence of the activity of the neuronal layers of the retina. All who have been dealing with the electrical response of the frog optic nerve know that even a little carelessness in the preparation of the eye is enough to abolish the slow potentials of this nerve without having any detectable effect on the ERG. This depends upon the high susceptibility of the neuronal layers to mechanical interference. The prepared retinas used by *Ottoson & Svaetichin* (1953) seemed to have their neuronal layers in a poor functional state, although the ERG response was obviously completely intact. This was apparently the

reason why they missed the neuroretinal potentials observed by the more careful japanese investigators *Tomita* and collaborators.

In their earlier works, *Tomita* and collaborators used large electrodes (tip diameter 7—20 μ) *which they first forced through the retina in order to make a channel through it, whereupon the measurements were made by moving the electrode back and forth in this preformed channel. Ottoson & Svaetichin* (1953) used microelectrodes having a tip diameter of less than 1 μ in their corresponding experiments, in which they localized the origin of the ERG. When measuring between an electrode in contact with the vitreous fluid and a less than 1 μ microelectrode introduced into the retina, practically no response was obtained before the electrode tip had perforated the external limiting membrane (*Brindley*'s R membrane).

However, withdrawing the microelectrode step by step, complex responses of large amplitudes were observed in all (!) retinal layers, due to current spread from the sources generating the ERG after damage to the retinal structures in the neighbourhood of the external limiting membrane. Considering this observation (*Ottoson & Svaetichin* 1952, 1953), it is understandable that the methods used by *Tomita* and collaborators in their earlier investigations (1950, 1952) were in our opinion not too convincing.

In our experiments we observed (*Ottoson & Svaetichin* 1953) that when the external limiting membrane had *once* (!) been perforated by the microelectrode, even if it was a small one having a diameter of less than 1 μ, it was then impossible to localize the sources of the ERG. Consequently, the recordings obtained by *Tomita* and co-workers in their earlier papers (1950, 1952) correspond to a mixture of slow neuroretinal potentials and ERG potentials of complex shape and polarity, caused by current spread through the damaged retinal structures in and close to the preformed channel through the retina. When a microelectrode having a tip diameter of 7—20 μ (used by *Tomita* and collaborators in their earlier papers) is forced into the retina from the vitreous side, heavy pressure must be exerted in order to perforate the internal and external limiting membranes, which necessarily results in damage of considerable retinal areas around the microelectrode channel.

The different types of experiments performed by *Ottoson & Svaetichin* (1953) in order to determine the structures producing the ERG,

unanimously favored the view that the ERG is a practically pure receptor potential.

In one type of experiment either the optic nerve response or the impulse activity of the *bipolar cells* was recorded simultaneously with the ERG. When the *exposed receptor* layer (!) or the vitreous side of the retina was treated by a cocaine solution, the ERG remained practically unchanged, whereas the nervous activity as recorded from either the bipolars or the optic nerve, was in a short time completely abolished. The only plausible interpretation of these experiments, as far as I can see, is that the ERG is generated by the receptor cells, on the activity of which cocaine seemed to have little or no effect.

In other types of experiments, in which the ERG and the activity of the optic nerve simultaneously were recorded, the retina was subjected either to temperature changes or to asphyxia. In both cases the optic nerve response proved to be much more susceptible than the ERG to the influences of temperature and asphyxia, and there is no reason to assume that the neurons in the retinal ganglion cell layer are more easily blocked by temperature changes or asphyxia than the bipolar cells.

The only logical conclusion that can be drawn is that the activity of the retinal bipolar and ganglion cells is more easily blocked by local anaesthetics, temperature and asphyxia than the activity of the rods and cones. Since the avtivity of the neuroretinal layers was shown to be blocked under these experimental conditions, although there were no significant alterations of the ERG recorded simultaneously, the ERG must necessarily be produced by the photoreceptors.

Components of the ERG.

The view that the ERG is a practically pure receptor potential, is further supported by the findings on fish single cones (*Svaetichin* 1953 a, and part II, this suppl.). The potentials obtained by intracellular recordings from single cones show a striking similarity to certain components of the ERG. Thus, the rectangular pulse-like action potential of the L type of cone agrees with the P_{III} component of the ERG, and the "on-off" response of the twin chromoreceptors corresponds well with the a-wave and the positive "off" effect respectively of the ERG.

The intracellularly recorded action potential of a single rod has not

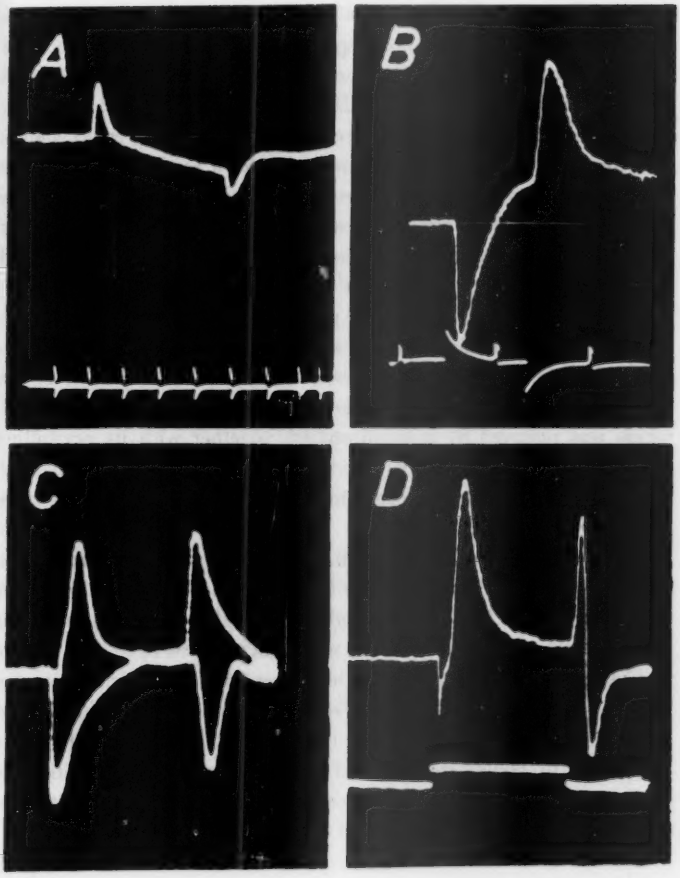

Fig. 1. (A) The ERG response of a well dark adapted fish eye. (B) The ERG response of a fish eye deprived of rods (perch). (C) Photographically superimposed rod and cone components of the fish ERG. (D) The ERG of a fish retina having both the rod and the cone layers intact.

yet been obtained but I assume that it is somewhat similar to the L cone response, the amplitude of the response, however, showing a decrease in the course of the illumination, corresponding to the process of adaptation (*Svaetichin* 1953 b). This suggestion is favored by observations made on intracellular recordings from certain insect photoreceptors (unpublished). The records showed a configuration similar to the ERG response of the rods seen in Fig. 1 A (see below). In the course of illumination the response was sometimes reduced to below the zero level (adaptation) although a minor "off" effect occurred showing a polarity opposite to that of the "on" response.

The c-wave of the ERG might be produced by the pigment epithelium (*Noell* 1954). This suggestion is supported by the findings of *Svaetichin, Fernández-Morán & Jonasson* (1956) of a component of the insect ERG, which they were able to record from the insect cornea, which was removed by microdissection, together with its crystalline cones and *pigment cells* (superposition eyes of giant moths). For this cornea-pigment cell preparation no photoreceptor elements have been described.

The type of recording obtained from a well dark adapted retina, having both the rod and the cone layers intact, is shown in Fig. 1 A (from *Svaetichin* 1953 a). The light stimuli of low intensity used for eliciting such a response from the well dark adapted eye (only b-wave and negative "off") were insufficient to evoke any response from the cones by intracellular recording. From a fish retina deprived of its rod layer, thus possessing only cones (Fig. 4, part I, this suppl.), it was impossible to record this kind of ERG response. Consequently, the experiments proved that the rod component of the ERG consists of a b-wave and a negative "off" effect (in the fish at least).

A fish retina having an exposed cone layer and no rods, showed either the type of response seen in Fig. 1 B (from *Svaetichin* 1953 b, perch), or a rectangular pulse-like response (P_{III}) or a mixture of both.

An ERG recorded from a fish eye having intact rod and cone layers is shown in Fig. 1 D, the light stimulus used being well above the threshold of the cones. Fig. 1 D illustrates a "synthesis" of the ERG made by photographical superimposing of two isolated rod and cone components of the fish ERG (perch).

In experiments on the fish eye (*Svaetichin* 1953 a) it was further

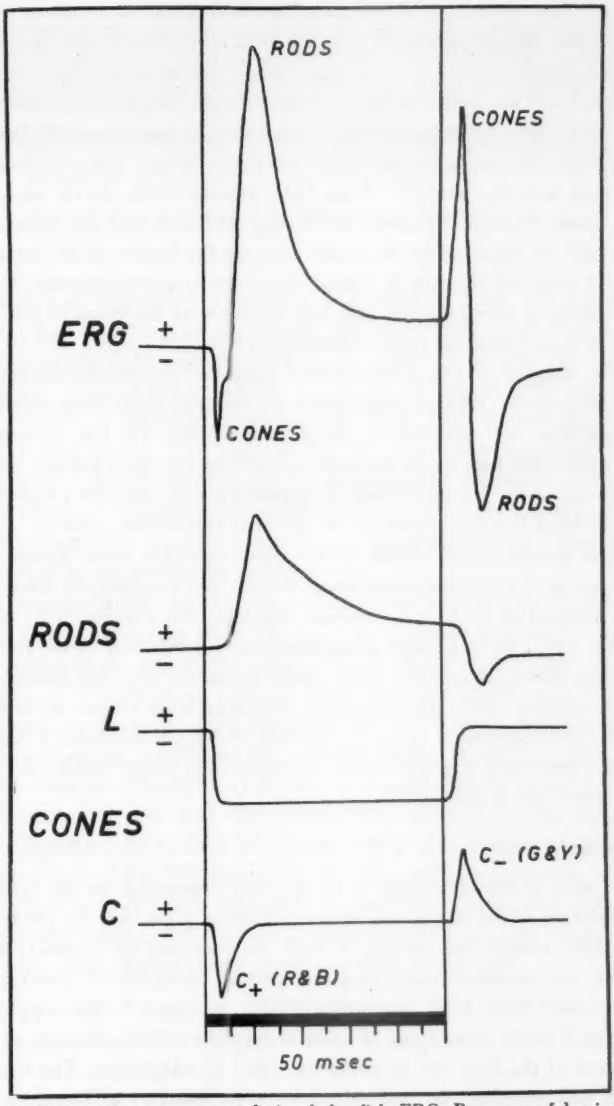

Fig. 2. Suggested component analysis of the fish ERG. Response of luminosity cones denoted by L. C is the response to white light of twin chromoreceptors.

shown that the a-wave of the fish ERG had a *constant latency independent of the intensity of the light stimulus*, and that this latency (15 msec.) was the same as that of the action potential obtained from a single cone, which response also proved to be independent (!) of the strength of the stimulus. On the other hand it was shown that the latency of the b-wave of the ERG was much longer (45—70 msec.), varying with the intensity of the light stimulus used. As far as I can see, these findings represent convincing evidences that the a-wave of the ERG is produced by the cones, whereas the b-wave is the response of the rods (in the fish at least). This view is also supported by experiments in which the fish and frog retinas were subjected to a stimulation by intermittent light (*Svaetichin* 1953 a).

The diagram in Fig. 2 represents a suggested component analysis of the ERG (fish), schematically drawn on the basis of the facts presented above. The "on" response of the chromoreceptors (C, Fig. 2) evoked by white light has, in intracellular recordings, the same polarity as the response of the L type of cone. In agreement with this, the a-wave and the isolated P_{III} component of the ERG are of the same polarity.

The positive "off" effect of the ERG recorded from retinas not possessing twin chromoreceptors, is smaller and is apparently caused by the rebound of the L cone response. Similarly, the negative "off" effect of the ERG, when present, is explainable as a rebound of the rod response. These suggestions are in good agreement with the findings of large positive "off" effects in ERG responses from retinas containing twin chromoreceptors (*e.g.* fish, tortoise, frog). On this basis a reasonable explanation is given for the division of the retinas in the "E" and "I" type (see *Granit* 1947).

Purkinje shift.

The shift of the maximum of the b-wave, depending on the state of adaptation of the retina, has been regarded as proof that the b-wave of the ERG reflects the activity of both the rods and the cones (*Granit* 1955, for literature concerning the spectral sensitivity of b-wave, see *e.g. Granit* 1947, 1955, *Svaetichin* 1953 b, and part V, this suppl.).

Fig. 3 shows recordings of spectral response curves obtained on the b-wave of the frog eye in different states of adaptation. The b-wave only was recorded, the "off" effect being excluded by technical means. The eye was from the beginning in strict scotopic state and was then

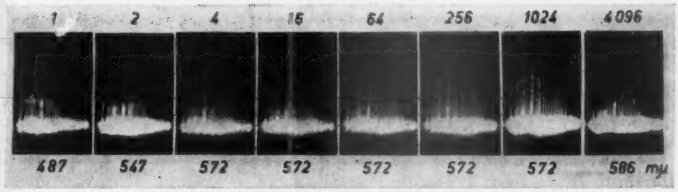

Fig. 3. Spectral response curves of the b-wave of the frog ERG. See text.

subjected to a gradually increasing adaptation to light. The amplification was kept constant throughout the experiment, whereas the intensity of the light stimulus used is indicated by the number above each set of recordings, and the maximum of the spectral response curve in mμ is indicated by the number below. (For the technique see part I, this suppl.) The spectral response curve showing a reduced amplitude (extreme right in Fig. 3) was obtained by use of a strong light stimulus, well above physiological limits (the light intensity used in this particular recording may have been even stronger than that indicated by the number above the record).

It is evident from the records in Fig. 3 that there was a continuous shift of the maximum when the eye was gradually adapted to light. According to the prevailing opinion the *Purkinje* phenomenon corresponds to a shift from scotopic rod vision to photopic cone vision. However, on the basis of the presented findings it is concluded that in strictly photopic conditions the rods are acting in parallel with the cones. Since no adaptation of the fish cones was detected when moderate illumination was used (*Svaetichin* 1953, and part II, this suppl.), the adaptation observed in the human eye in photopic state seems to be an effect of the light adapted rods. The findings presented above agree well with the view suggested by *Dartnall* (1948), that changes which take place in the rhodopsin system in the course of adaptation are responsible for a spectral sensitivity shift of the rods.

REFERENCES

Bernhard, C. G., Acta Physiol. Scand. 1942. *3*. 301.

Brindley, G. S., J. Physiol. 1956. *134*. 339.

— J. Physiol. 1956. *134*. 353.

— J. Physiol. 1956. *134*. 360.

Dartnall, H. J., Brit. J. Ophthal. 1950. *4*. 23.

Granit, R., Sensory Mechanisms of the Retina. Oxford Univ. Press. 1947.

— Receptors and Sensory Perception. Yale Univ. Press. 1955.

Kühne, W. & J. Steiner, Unters. physiol. Inst. Heidelb. 1880. *3*. 327.

— Unters. physiol. Inst. Heidelb. 1881. *4*. 64.

Noell, W. K., Am. J. Ophthal. 1954. *38*. 78.

Ottoson, D. & G. Svaetichin, Cold Spr. Harbor Symposia Quant. Biol. 1952. *17*. 165.

— Acta Physiol. Scand. 1953. *29*. Suppl. 106. 538.

Schubert, G., Graefes Arch. Ophthal. 1953. *154*. 125.

Svaetichin, G., Acta Physiol. Scand. 1953 a. *29*. Suppl. 106. 565.

— Acta Physiol. Scand. 1953 b. *29*. Suppl. 106. 601.

Svaetichin, G., H. Fernández-Morán & R. Jonasson, Abstracts of XXth International Physiological Congress. 1956. 866.

Tomita, T., Jap. J. Physiol. 1950. *1*. 110.

Tomita, T & A. Funaishi, J. Neurophysiol. 1952. *15*. 75.

Tomita, T. & Y. Torihara, Jap. J. Physiol. 1956. *6*. 118.

V. THE CONE FUNCTION RELATED TO THE ACTIVITY OF RETINAL NEURONS

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Numerous attempts have been made to attack the color vision problem by electrophysiological means. In experiments based on ERG recordings it was possible to show differences in the spectral sensitivity corresponding to the scotopic and photopic visibility curves (*Himstedt & Nagel* 1901, *Piper* 1904, 1905, *Brossa & Kohlrausch* 1913, *Chaffee & Hampson* 1924, *Graham, Kemp & Riggs* 1935, *Graham & Riggs* 1935, *Granit* 1937, *Granit & Munsterhjelm* 1937, and *Granit & Wrede* 1937). However, no signs of the chromoreceptor mechanisms were obtained using these methods.

Developing a technique for microelectrode recording of the spike activity from single neurons in the retinal ganglion cell layer (frog), *Granit* and *Svaetichin* (1939) obtained narrow band spectral sensitivity curves, their maxima being distributed in three main regions, *viz:* 460—480 mμ, 500—530 mμ, and 580—600 mμ. These narrow band spectral sensitivity curves, later called "modulators" (*Granit* 1943), were supposed to reflect the chromoreceptor mechanisms. Since there were three predilected regions for their occurrence in the spectrum, the findings were considered to support the trichromatic theory.

Dominator—Modulator Theory.

Granit and collaborators later extended similar studies to a large number of different species of vertebrates (see reviews by *Granit* 1947, 1950, and 1955), and on the basis of these findings *Granit* (1943) postulated his dominator—modulator theory.

According to *Granit* (*e.g.* 1943, 1945, 1947, 1950, 1955), the neurons ("modulator elements") from which the narrow band spectral sensitivity curves were recorded, are supposed to mediate the signals for chromaticity whereas other neurons, into which the signals from the three types of modulator elements are believed to converge, produce broad spectral sensitivity curves. The latter neurons, being called "dominator elements" by *Granit,* are supposed to mediate the signals for the photopic luminosity. In the course of dark adaptation the photopic dominator may change to a scotopic dominator in analogy with the *Purkinje* phenomenon.

Receptor Potentials — Neuronal Spikes.

On the basis of the present knowledge of the function of single cones it is of interest to compare the spectral response curves of single cones with the spectral sensitivity curves obtained using the spike activity recorded from individual neurons in the retinal ganglion cell layer as index (below called "the retinal spike method").

It is important to analyze the spike response pattern recorded from the retinal neurons in relation to the properties of the receptor responses of individual cones. Working on single optic nerve fibers, separated by microdissection from the optic nerve fiber layer of the frog retina, *Hartline* (1938, 1940) observed three main types of spike responses: (1) 50 per cent of the recorded responses were pure "on-off" responses, (2) 30 per cent responded only at "off", and (3) 20 per cent of the records showed an initial burst of impulses succeeded by a short silent period followed by a low frequency firing, continuing as long as the light stimulus lasted. Substantially, the same features of the retinal ganglion cell discharges were found also in other vertebrates investigated. However, the existence of a pure "off" effect seemed doubtful (*Granit* 1955).

Studying the spectral sensitivity of the pigeon with the retinal spike method, *Donner* (1953) reports that all the responses to the spectral stimuli were pure "on-off" responses.

Thus, on the basis of the information obtained, either by investigating the activity of dissected single nerve fibers or by microelectrode recordings from the retinal ganglion cells, it appeared that the impulse discharges of the neurons in the retinal ganglion cell layer were restricted primarily to "on" and "off" of the light stimulus.

On the other hand, we know from the studies of the cone action potential (part II, this suppl.) that the chromoreceptors and the luminosity cones as well, responded with a square wave response, continuing as long as the light stimulus lasted. Further, it was shown that the chromoreceptors produced "on-off" responses only if they were subjected to a colorless stimulus, or if they were stimulated by spectral light of the wavelengths close to the neutral point of the spectral response curve of a cone pair.

Considering these facts, *Donner*'s report (1953) of exclusively "on-off" responses, which should correspond to the maxima of the spectral response curves of the chromoreceptors, is rather confusing.

Concerning the human vision, I am aware of the work done by *Ratliff* (1952), *Ditchburn* & *Ginsborg* (1952, 1953), *Riggs, Ratliff, Cornsweet* & *Cornsweet* (1953), and *Riggs, Armington* & *Ratliff* (1954) showing: (1) the existence of motions of the retinal image during fixation, and (2) the fading out of the contours of steadily fixated small test objects. Owing to particular experimental conditions in the above mentioned studies, the image on the retina remains stable regardless of the continuous involuntary eye tremor. Under these artificial circumstances the "on-off" response pattern has no chance to be evoked, and it appears reasonable to assume that this lack of "on-off" responses has something to do with the reported phenomena.

Obviously, "on" and "off" responses of the receptors can be evoked solely in the case of the light stimulus being switched on or off. Continuously looking against an evenly illuminated colored or colorless wall, our visual perceptions do not change either in respect to luminosity, hue or saturation. Under these circumstances there are no possibilities to start any "on-off" patterns, as far as I can see. Consequently, there must be continuously firing neurons signalling luminosity, hue and saturation in the course of the light stimulus!

Retinal Ganglion Cells.

For the interpretation of the extensive material collected by recording the activity of neurons in the retinal ganglion cell layer, it is important to know: (1) the different types of retinal ganglion cells and their relations to the rods and cones, and (2) whether the responses recorded from the retinal ganglion cells represent the activity of all known types of neurons in the retinal ganglion cell layer, or whether they are restricted to some particular types of ganglion cells.

The types of neurons in the simian and human retina are particularly well known from the work of *Polyak* (1941, 1949). The retina of the higher diurnal vertebrates is, in principle, similarly built (*e.g. Ramón y Cajal* 1894, 1933, *Walls* 1942, *Polyak* 1941, *Detweiler* 1943, *Prince* 1956). The retinal ganglion cells of the primate and simian retina (*Polyak* 1941) are divided into two main groups: (1) five types of diffuse ganglion cells with large cell bodies and extensive dendritic expansions, making synaptic contacts with a large number of bipolar cells, and (2) monosynaptic or individual ganglion cells, which are the smallest and most numerous of all, represented by the midget gangli-

on cell type. The dendritic tree of the midget ganglion cell is small enough to make individual synaptic connection with the midget bipolar cell (*Cajal*'s "cone bipolar"). In the simian and human retina the cell body of the midget ganglion cell has a diameter well below 20 μ (10—18 μ), whereas the types of diffuse ganglion cells reach a diameter of 40—50 μ. In the avian retina these ganglion cells are even smaller (*Ramón y Cajal* 1894, 1933). For instance, in the retina of the pigeon the small ganglion cells corresponding to the midget ganglion cells are only about 7—8 μ in diameter, and the largest of the diffuse types of ganglion cells have a diameter of about 17 μ (*K. Engström* 1956, personal communication).

Concerning the relation of the rods and cones to the different types of ganglion cells, histological evidences show (*Polyak* 1941) that the diffuse type of medium and large ganglion cells are related to both rods and cones, and that they cover wide retinal areas, whereas the midget ganglion cells have individual synaptic relations to the midget bipolars ("cone bipolars"), which are exclusively connected to the cones. According to *Polyak* (1941 p. 418): "the midget ganglion cell (s) is the only or, at any rate, the principal neuron variety concerned with the color perception", and further "What role in the perception of colors, *if any*, the diffuse ganglion cells play — —".

Consequently, from histological evidences to conclude, the midget ganglion cells, *i.e.* the smallest type of neurons in the retinal ganglion cell layer, are the ones reflecting the activity exclusively of the cones which are responsible for the color vision, whereas the neurons of large and medium size (the diffuse ganglion cells) mediate the activity of less restricted retinal areas and of both rods and cones.

Recording Conditions.

It is important to know whether it is possible, with the methods hitherto used, to obtain responses from all types of neurons in the retinal ganglion cell layer, or whether there are some restrictions due to technical reasons.

Obviously, when preparing single optic nerve fibers having a maximal diameter of about 5 μ it is possible to use only the largest ones. Hence, the types of spike responses described by *Hartline* (1938) certainly represent the activity of the large and middle sized neurons.

Rushton (1949, 1950, 1953), working in *Granit*'s own laboratory,

showed that the individual large spikes recorded with 25 μ micro-electrodes from the cat retina were obtained from large ganglion cells 30—50 μ in diameter. Concerning this observation *Granit* (1955 p. 135) writes: "It is often stated (*cf. Wald* 1953) that all my records were from a specific type of isolated giant ganglion cell. This is erroneous." However, in 1950 (p. 41) *Granit* writes: "two types of spike can be recorded by the platinum wire micro-electrode from the cat's eye: a small spike which need not concern us here because these spikes cannot be maintained well enough for the time necessary for analytical work, and a considerably larger spike. We have only used the large spike which ranges in size from 0.15—0.30 mV".

The general observation that the retinal ganglion cell spikes are primarily restricted to "on" and "off", and in particular *Donner*'s finding (1953) of exclusively "on-off" responses to spectral stimuli, supposed to reflect the chromoreceptor mechanisms in pigeon, are inconsistent with the continuous discharge expected from the properties of the receptor responses of single cones. These observations favor the view that the midget type of ganglion cell, or corresponding type, which reflects the activity of the cones proper, has not at all been reached with the recording techniques hitherto used!

The type of metal microelectrode originally used by *Granit* and *Svaetichin* (1939) in their work on the frog retina (diam. 2—15 μ), was developed by myself in 1937 and used at that time for unpublished work on tissue cultures of chicken spinal ganglion cells. Later in Stockholm, *Granit* used an "improved type" of electrode and found that he got the best results with the electrodes having a diameter of 25 μ. Using his 25 μ microelectrode on the pigeon's retina, *Granit* (1942 p. 118) writes: — "I never have succeeded in obtaining a restricted response from this retina with the same microelectrodes which have given single spikes from eyes of rats, guinea pigs, and cats". In the Silliman Memorial Lecture (1955) *Granit* writes (p. 137): "Recently, *Donner* (1953), with his improved microelectrodes, has measured the sensitivity distribution of individual elements in the pigeon's eye", (*Donner* used a type of low-resistance microelectrode "described by *Svaetichin* 1951"). This proves that with the 25 μ microelectrode it was impossible (*cf.* above and *Granit* 1942) to obtain individual recordings even from the largest (17 μ) ganglion cells in the pigeon retina.

Concerning the relation between the size of the cell body and the

amplitude of the recorded extracellular action potential, I have experiences from investigations on single spinal and autonomic ganglion cells (*Svaetichin* 1948, 1951). In these experiments the microelectrode recordings were carried out under simultaneous microscopic inspection. the cell borders being made visible by staining. It was observed that the amplitude of the extracellularly recorded action potential was approximately proportional to the volume (cell diameter^3) of the perikaryon. From these experiments I also observed that recording of action potentials by an extracellular microelectrode from myelinated single axons was next to impossible, if the preparation was not very dry. Hence, I suppose that all the recordings done with extracellular microelectrodes from the retinal ganglion cell layer, mainly represent the activity of the cell body and the initial segment, particularly considering that the optic nerve fibers are surrounded by the vitreous fluid, and that they are maximally 5 μ in diameter. This is in agreement with the view of *Barlow* (1953 a).

In his work on retinal ganglion cells in cat, *Kuffler* (1953) used metal microelectrodes 5—15 μ in diameter, and obtained spikes having a maximal amplitude of about 600 μV. Working on the pigeon's retina, *Donner* (1953) got individual responses of 50 μV maximal amplitudes, and *Barlow* (1953 b) obtained action potentials of 30—80 μV amplitude in the frog. In the cat the perikarya of the largest retinal ganglion cells have a diameter of about 50 μ, in the pigeon they are about 17 μ, and in the frog 20 μ. Apparently, the highest impulse amplitudes correspond to the largest cells. Thus, the rule concerning the relation between the cell diameter and the spike amplitude found on the spinal ganglion cells seems indeed very roughly to be valid for the retinal ganglion cells too.

On the other hand, the smallest retinal ganglion cells in the pigeon retina were shown to have a cell body diameter of about 7—8 μ. Actually, the perikarya of the small ganglion cells in the pigeon retina have about the same diameter as the bipolar cells and the cones themselves (the cones being longer). From a neuron having a cell body with a diameter of about 7—8 μ we can expect to get, by extracellular recording, an impulse with an amplitude below the noise level of the amplifier.

In order to record from the midget ganglion cells (or corresponding small ganglion cells) an extracellular response, the electrode must be

optimal in respect to size, resistance, and its location close to the cell body. When recording from the retinal ganglion cell layer with an electrode larger than about 5 μ, the tip of the electrode is placed on the vitreous side of the internal limiting membrane, since this membrane has a considerable mechanical strength and is impossible to penetrate without pressure and damage to the cells below. (*Ottoson* & *Svaetichin* 1953). Thus, the measurements hitherto made from the retinal ganglion cell layer are recorded across the external limiting membrane and the layer of the optic nerve fibers. These recording conditions do not improve the possibilities of reaching the perykaryon of the midget type of ganglion cell.

Using low resistance metal microelectrodes (1—8 μ diam.) introduced into the bipolar layer from the exposed receptor side, or from the vitreous side, *Ottoson* and *Svaetichin* (1953) succeeded in getting spike recordings from the bipolar cells in the frog retina. This proved to be a very difficult task, although the largest bipolars in frog are about 13 μ in diameter. We succeeded only in a few occasions to get recordings of a low amplitude. For additional proof of the difficulty experienced in obtaining impulses from neurons of this size, it is worth mentioning that *Tomita* and his co-workers (1950, 1952), using 7—20 μ electrodes, denied the existense of spikes in the bipolar layer.

On the basis of the activity recorded from dissected optic nerve fibers (*Hartline* 1940) and microelectrode recordings from retinal ganglion cells (*Barlow* 1953 b), the receptive field determined (200—600 μ diam.) is large and corresponds well to the field covered by the diffuse ganglion cells. The frog has also small ganglion cells (*Cajal* 1894), which are certainly connected to a very restricted number of cones — in order to achieve detail vision. From the relation between the number of optic nerve fibers and the number of receptors it can be concluded that there must be small ganglion cells with receptive fields much smaller than 200 μ (*cf. Barlow* 1953 b), but these small ganglion cells have not been reached by the techniques previously used.

Thus, all the facts presented above support the view that the recordings hitherto made from the retinal ganglion cell layer, represent the activity of the large and medium sized neurons, *i.e.* the diffuse types of retinal ganglion cells, the midget type (or corresponding type) of ganglion cell being missed.

From the histological studies to conclude (*Polyak* 1941), the midget

ganglion cells make individual synaptic connections with the midget bipolars (*Cajal*'s cone bipolars), which are exclusively connected to the cones. Hence, it is probable that the midget ganglion cells mediate the signals from the chromoreceptors, and this signal is expected to be a steady discharge, continuing as long as the chromatic light stimulus lasts.

Further, it is reasonable to assume that the "on-off" responses, evoked when the chromoreceptors are stimulated by achromatic light, are added to the luminosity signals carried by the large diffuse type of retinal ganglion cells.

Additionally, it is suggested that a continuous spike signal reflecting the activity of the individual luminosity type of cones (L) is also mediated by a certain midget type of ganglion cell, corresponding to the detail vision in the central retinal areas. This signal possibly is the only one carried by the small ganglion cells in animals without color vision.

SPECTRAL SENSITIVITY AS OBTAINED ON RECEPTORS AND GANGLION CELLS

In the following, the relation is analyzed between the spectral response curves of single cones and the spectral sensitivity curves obtained on the retinal ganglion cells, trying to fit together the results obtained with the different methods.

In order to achieve a general survey of the dominator and modulator curves obtained by the retinal spike method on different animals, the curves in Fig. 1 have been redrawn, representing typical curves from *Granit*'s work on: fish (1941), tortoise (1941), grass-snake (1943), rat (1941), guinea pig (1942), and cat (1943).

FISH.

To begin with, a comparison is made with the nearest equivalent to the single cone studies, *viz.* the investigations by *Granit* on the fish retina (*Cyprinus, Tinca, Anguilla, Granit* 1941). The curve denoted by "S" in Fig. 1 a (continuous line) represents the average curve of microelectrode recordings from dark adapted tench, and the curve "P" is the corresponding average curve obtained on light adapted tench

(continuous line with dots, Fig. 1 a). Several individual curves obtained with the retinal spike method on the light adapted tench were presented by *Granit* (1941); however, their maxima coincide with the average maximum at about 600 mμ, with the exception of one of them which has been redrawn and indicated by "I" (dotted line, Fig. 1 a).

At bottom of Fig. 1 a, the letters with associated arrows denote the maxima of the spectral response curves recorded from single cones in fish. The two arrows at "L" show the limits for the scattering of the L maxima. The arrows at "Sg" and "Pg" give the mean maxima of the scotopic and photopic luminosity curves respectively, obtained by *Grundfest* (1931, 1932) on the living sunfish.

It appears that the crests at about 540 and 600 mμ of the average curves, "S" and "P", obtained with the retinal spike method, agree rather well with the maxima of the scotopic "Sg" and photopic "Pg" luminosity curves respectively, obtained by *Grundfest*. On the other hand, it is seen that the crest of the photopic luminosity curve "Pg" obtained on the living sunfish, coincides approximately with the maximum of the spectral response curve of the L type of cone. It is reasonable to assume that the curve obtained with the ganglion spike method on the light adapted tench reflects the activity of the L type of cone (and possibly additionally that of light adapted rods, *Svaetichin* 1953 b, and part IV, this suppl.).

In accordance with the findings obtained on single cones of fish, there must be certain retinal ganglion cells in this animal which reflect the activity of the chromoreceptors B, G, R and Y respectively. However, the spectral response curves obtained with the retinal spike method by *Granit* on the fish eye, do not reflect the activity of the chromoreceptors. The occasionally found curve ("I" dotted line, Fig. 1 a) has hardly any connection with the chromoreceptor mechanisms, since otherwise the activity of the other types of cones should have been observed too.

Certainly, *Granit* started the investigations on the fish eye in order to find chromoreceptors, and surprisingly enough, only responses corresponding to the scotopic and photopic luminosity mechanisms were found. Of course, in an animal with color vision there must be particular retinal neurons delivering the signals for hue to the higher centres. The neurons which mediate the chromaticity responses, were apparently not reached with the microelectrode technique used by *Granit*. Conse-

quently, a comparison of the results achieved with the two different methods proves that the microelectrode recordings from the retinal ganglion cell layer in fish are obtained exclusively from the diffuse types of ganglion cells mediating signals for scotopic and photopic luminosity.

TORTOISE, SNAKE.

By aid of the retinal spike method *Granit* also investigated the retina of the tortoise *(Testudo graeca)* and in Fig. 1 b the continuous line with dots gives the average photopic dominator curve (*Granit* 1947) redrawn from *Granit*'s paper (1941), and the continuous line denotes the average "red receptor" curve presented by *Granit* (1940). In *Granit*'s publication (1941) several individual curves were presented, showing almost the same configuration as the average curves; all the maxima being situated at about 610—620 mμ. The only exception is the "once found" individual curve "I" shown by the dotted line in Fig. 1 b.

The average as well as the individual curves obtained on the tortoise agree with the photopic luminosity maxima of the fish (*Grundfest*'s curves, *Granit*'s own curves, the L cone). Further, in "Dressur" experiments (*Vojtusiak* 1932, see the survey by *Walls* 1942) it has been shown that the photopic maximum of the turtle is in the orange region (about 600 mμ). Thus, it appears reasonable to consider both the average and the individual curves obtained on the tortoise as curves reflecting the activity of the luminosity mechanism (L type of cone).

The significance of the "once found" curve is uncertain ("I", dotted line Fig. 1 b), but it could possibly reflect the activity of the few rods in the retina of the tortoise. The hump seen on the average curve in the same region (continuous line with dots Fig. 1 b) might be explained in a similar fashion; or the hump possibly corresponds to a submaximum of the L type of spectral response curve (part II, this suppl.).

The retina of the tortoise contains both double and single cones (*Walls* 1942, *Prince* 1956) and the animal has a well developed color vision with a closed color circle similar to that of fish or human (see the survey by *Walls* 1942). Nevertheless, the retinal spike method again failed to bring forth spectral sensitivity curves corresponding to the chromoreceptors. The types of large ganglion cells in the retinal ganglion cell layer, reached by the microelectrode used in these studies, reflected solely the luminosity mechanisms.

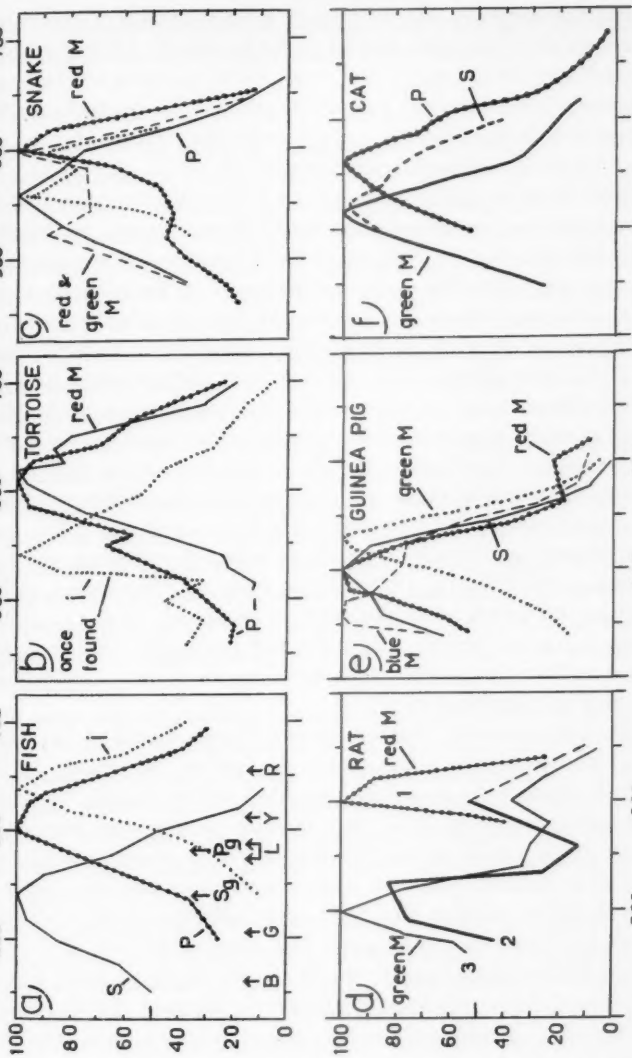


Fig. 1. Dominator and modulator curves redrawn from different publications by *Granit*. Full description in text.

The next diagram, Fig. 1 c, shows spectral sensitivity curves obtained by the retinal spike method on the grass-snake (*Tropidonotus*). According to *Granit*'s view (1943, 1947, 1955), the curve with the crest at about 560 mμ (line with dots) is the photopic "dominator", and the curves with their maxima to the right and to the left of that curve are "red and green modulator" curves respectively.

With the present background in mind, I am inclined to offer another interpretation of the presented curves which are grouped around two main maxima, *viz.* that they reflect the photopic and scotopic mechanisms of the retina. The suggestion is founded on the following facts: (1) in fish and tortoise the retinal spike method could not reach the ganglion cells delivering the signals for chromaticity, and scarcely does so in the snake either, (2) the crest at about 600 mμ agrees with the value of the photopic luminosity mechanisms found in fish and tortoise, and the crests towards the short wave end of the spectrum could well correspond to the scotopic ones, (3) on the basis of the findings of mutual exclusiveness of the red and green receptor responses in fish cones, and also generally speaking, it is hard to believe that the same ganglion cell would deliver signals for both red and green, and (4) *Granit* writes (1943, p. 109): "To be true, the green region of the spectrum has sometimes become more prominent later in an experiment when the animal has been lying in the dark box and its eye only illuminated by threshold stimuli. The pure cone eye of the Greek tortoise behaved in a similar fashion".

The tortoise and the grass-snake as well, are claimed to have cone eyes; the histological differentiation is, however, not certain. In the tortoise retina the rods are also described, and concerning the snake histologists say (*Walls* 1942) that the cones are developed from rods! *Walls* (1942 p. 611) writes: "Prior to 1877, about everyone who described a chelonian retina saw rods in it, but since that time, owing to one of the few mistakes (and the weighty authority) of *Max Schultze*, the turtles have been placed among pure-cone reptiles".

The ERG of the tortoise (*Testudo graeca*) displays a small b-wave (*Bernhard* 1941), which further proves the presence of rods (*Svaetichin* 1953 b). The ERG of the grass-snake too has a b-wave, according to the few experiments I have carried out.

Further, there are nocturnal species among snakes and tortoise which are described as having cone eyes. Thus, the question of the "pure"

cone retina is far from settled. Possibly, receptors which histologically have cone properties, may functionally be rod like.

MAMMALS.

In his work on the rat (1941), guinea pig (1942), and cat (1942 b) *Granit* observed that the spikes obtained were much larger in mammals than in the other vertebrates earlier investigated, *viz.* frog, fish, tortoise, snake, pigeon. This is apparently due to the existence of larger cell bodies in the diffuse types of retinal ganglion cells in the mammals investigated. *Granit* also found that his 25 μ type of microelectrode was satisfactory in obtaining spikes from individual neurons in these mammals. All retinal work published by *Granit* since then has been done on the cat, because on this animal it was easier to get large responses from individual neurons.

The significance of *Granit*'s findings of modulators (indicating chromoreceptors) in the cat, the animal on which the main part of his work is based, is difficult to understand, considering that it has been shown that the cat is color blind (*e.g. Meyer, Miles* & *Ratoosh* 1954, see also the surveys by *Walls* 1942, 1953). Further, the spectral sensitivity as reflected in the impulse pattern records from the visual central pathways, agrees well with a luminosity curve (rhodopsin), giving no support for the existence of chromoreceptor mechanisms (*Ingvar* 1956, *Cohn* 1956).

Granit's spectral sensitivity curves for mammals have been redrawn in Fig. 1 d—f. The diagram in Fig. 1 d shows the "green" and "red" modulators of the rat according to *Granit*'s interpretation. The curve (1) was obtained after light adaptation of the retina, and the curves denoted by (2) and (3) show the subsequent changes in the spectral sensitivity of the same "element" in the course of the following dark adaptation.

From the histological studies to conclude, the diffuse type of large ganglion cell covers large retinal areas, receiving information from both rods and cones. The changes in the spectral sensitivity evoked by adaptation, as seen in Fig. 1 d, seem to reflect the activity of cones and rods as well. It appears to me that the spectral sensitivity curves in Fig. 1 d nicely show how the activity of the limited number of cones in the rat retina is depressed in the course of the dark adaptation. Further, it seems generally unreasonable to assume that the signals for green and

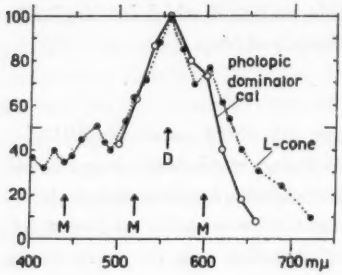


Fig. 2. Photopic dominator curve of cat redrawn from *Granit* 1945, indicated by continuous line and open circles. L type of spectral response curve of fish cone, given by dotted line and filled circles, is shifted by 20 mμ towards the short wave end of the spectrum.

red (if any in the rat) should be carried by the same neuron, particularly considering the mutual exclusiveness of the receptor potentials for the opponent color pairs, as shown in the fish retina.

Similarly, the spectral sensitivity curves obtained on guinea pig and cat (Fig. 1 e, f) seem to reflect different states of adaptation of the rods, mixed with the signals for photopic luminosity from the cones. Apparently, all records are obtained from the diffuse type of large retinal ganglion cells, which mediates the activity of both rods and cones. This interpretation of *Granit*'s findings agrees with that of *Walls* (1953).

The hump seen at about 600 mμ on the photopic dominator curve obtained on the cat (continuous line with dots "P" in Fig. 1 f) apparently gave *Granit* the idea that the photopic dominator is composed of the modulator curves. A further support for this was found in the fact that also the photopic luminosity curve of humans shows a corresponding hump (*e.g. Wright* 1946). However, it has been shown (*Wright* 1952) that humps appear as clearly on the luminosity curve obtained on totally color blind cone monochromats too. It is interesting to note that the hump seen on the photopic dominator curve of cat (continuous line, open circles, Fig. 2, redrawn from *Granit* 1945) agrees well with one of the submaxima of the spectral response curve of the L type of fish cone (dotted line and filled circles, Fig. 2). The L type of spectral response curve has been shifted about 20 mμ towards the short wave end of the spectrum in order to get the maxima of the curves to coincide. "M" indicates the modulator maxima, and "D" the maximum of the photopic dominator in cat (*Granit* 1945, 1955).

Trying to split the photopic dominator curve of cat in the modulator

components, extensive work was done by *Granit* (1945, 1947, 1950, 1955) using a method of selective bleaching. By this means he apparently succeeded in producing small alterations in the photopic dominator curves and, subtracting these new curves from the original dominator curves the modulators for the cat were achieved.

Since the cat has been proved to be color blind, there are no reasons to expect the existence of chromoreceptors in this animal. The photopic dominator of the cat agrees to some extent with the (20 mμ shifted) spectral response curve of the L type of fish cone (Fig. 2), which cone apparently is responsible for the luminosity mechanism. Hence, it seems reasonable to suggest that the obtained alterations of the photopic dominator curve of cat were caused by selective bleaching of some of the component photopigments which the L type of cone appears to contain (part II, this suppl.).

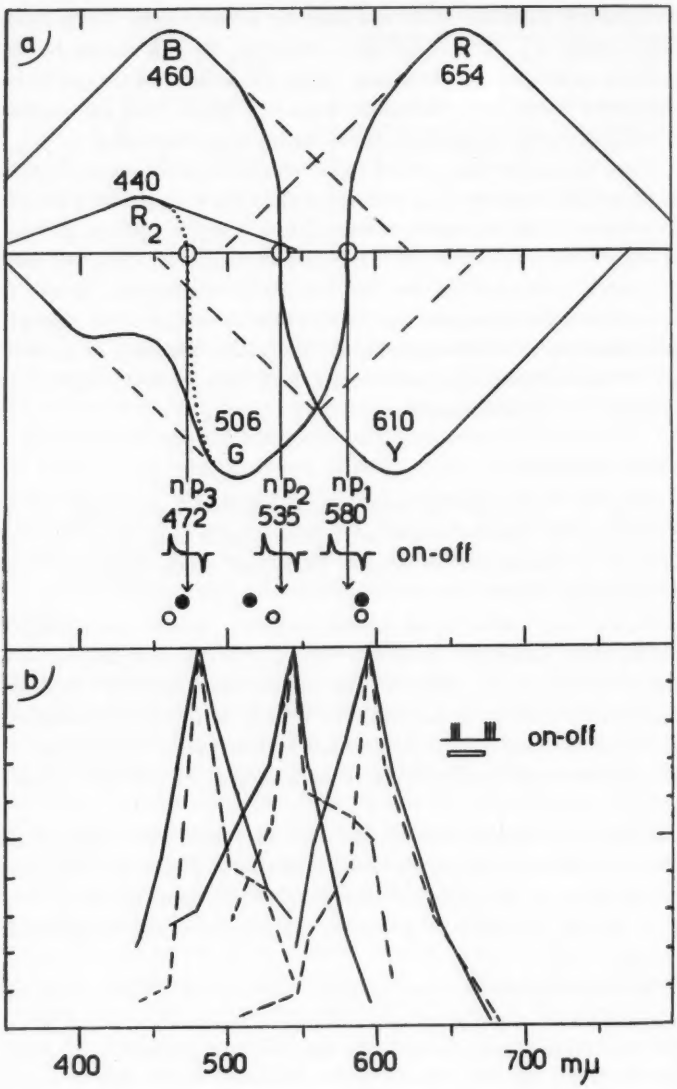
FROG, PIGEON.

Against the background of the single cone studies, I am finally trying to analyze the results obtained by the retinal spike method on the frog (*Granit* & *Svaetichin* 1939, *Granit* 1941) and on the pigeon (*Granit* 1942, *Donner* 1953).

On the frog, narrow band spectral sensitivity curves were obtained, having their maxima between 450—600 mμ in the spectrum, but also broader curves were obtained. The narrow band spectral sensitivity curves were considered to reflect the activity of the chromoreceptors, and since they appeared to be grouped in three preferential regions of the spectrum, this was believed to be a support for the trichromatic theory.

Similar "modulator" curves, but even narrower, were obtained by *Donner* (1953) on the pigeon retina (redrawn in Fig. 3 b). They were also grouped in three preferential regions of the spectrum; hence, they now seemed definitely to prove the correctness of the trichromatic theory.

The most common type of curve obtained by *Donner* from the pigeon's retina, was a broad curve having its sensitivity at about 580—590 mμ. These broad curves closely resemble the "photopic dominator" curve, which was the only one *Granit* obtained in his studies on the pigeon's retina with the 25 μ microelectrode (not on individual responses). An average curve of three individual photopic dominator


a
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R
654
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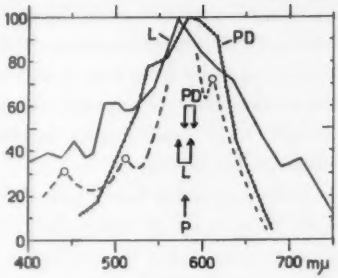

Fig. 4. Average photopic dominator curve obtained by *Donner* (1953) on pigeon, given by continuous line with dots; limits for scattering of maxima shown by arrows at "PD". Heavy line represents L type of spectral response curve obtained on fish cone; arrows at "L" give scattering of maxima. Maximum of photopic luminosity curve obtained on fowls by the "Dressur" method, denoted by "P" (*Honigmann* 1921).

curves, which in *Donner*'s recordings always showed several humps, has been redrawn in Fig. 4 (continuous line with dots, arrows at "PD" give limits for scattering of maxima). The open circles joined by the broken line in Fig. 4 denote the submaxima found by *Donner* to occur in "elements with a more complicated sensitivity curve".

The curve given by the heavy line in Fig. 4, represents the L type of spectral response curve obtained on fish, in which animal the L cone constitutes the photopic luminosity mechanism (arrows at "L" indicate limits for scattering of maxima). The crest of the photopic luminosity curve of fowls obtained by the "Dressur" method, is denoted by the arrow at "P". These findings are in agreement with the interpretation that the photopic dominator curve obtained on the pigeon and the L type of spectral response curve obtained on fish, both reflect the luminosity mechanism. Further, the luminosity mechanism in both fish and bird is apparently based on the existence of a particular L type of cone. The maximum of the pigeon's L type of cone is shifted (as compared with the fish L cone) about 10 mμ towards the long wave end of the spectrum. Both curves show submaxima, apparently due to the presence of several photopigments in the outer limb of the L type of cone.

On the basis of the remarkably good agreement between the "photopic dominator" curve obtained by the retinal spike method and the L type of spectral response curve recorded from a single cone, one would,

Fig. 3. Spectral response curves of fish chromoreceptors at (a) and modulator curves obtained by *Donner* (1953) on pigeon at (b). Filled circles (*Granit* & *Svaetichin* 1939) and open circles (*Granit* 1941) on top of pigeon modulator curves indicate average maxima of frog modulator curves. Neutral points of fish chromoreceptors denoted by np_{1-3} and wavelengths in mμ.

of course, expect a good agreement between the spectral response curves of the chromoreceptors and the "modulator curves" too. However, as appears from Fig. 3, this is not at all the case. In this diagram the average spectral response curves recorded from the chromoreceptors of fish, have been drawn together with *Donner*'s modulator curves obtained on pigeon.

In the fish retina four types of chromoreceptors were found, whereas only three types of "modulator curves" were obtained on frog and pigeon. Particularly the maximum of the spectral response curve of the R cone is situated too far away from the "modulator curve" to be explained by experimental errors.

The open circles on the top of the pigeon modulator curves give the average values of the maxima obtained by *Granit* (1941) on the frog, and the filled circles indicate the average values reported for frog by *Granit* and *Svaetichin* (1939).

The striking resemblance between properties of the visual system of fish and bird is not only restricted to the luminosity mechanism, since it has been shown that both animals have a well developed color vision which, when compared with our own, is essentially identical. Birds have been proved to distinguish four colors: blue, green, yellow, red, and further, simultaneous color contrast phenomena in respect of the color pairs red-green and yellow-blue have been shown to exist in birds too (see the reviews of *Walls* 1942 and *e.g. Honigmann* 1921, *Blässer* 1926, *Lashley* 1916, *Watson* 1915, *Revész* 1921, *Hamilton* & *Coleman* 1933, *Bailey* & *Riley* 1931, and *Path* 1935). Consequently, we are justified in expecting similar properties of the chromoreceptors in both fish and bird (—and in the human as well).

A rather good agreement exists between the data of the modulator curves obtained on the frog and those obtained on the pigeon (Fig. 3). However, the frog modulator curves, as compared to those of the pigeon, being shifted (about 10 mμ) towards the short wave end of the spectrum.

The dominator curves obtained from the pigeon's retina are too narrow to correspond to the spectral response curves of the chromoreceptors. In order to demonstrate this fact a mean cone spectral response curve has been drawn in Fig. 5 (continuous line) together with one of *Donner*'s "modulator curves" (heavy line). The broken line represents the narrowest conceivable computed spectral sensitivity curve.

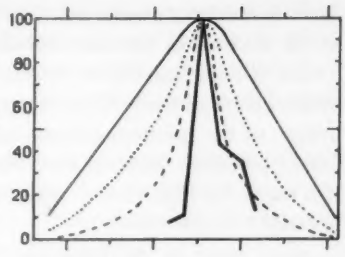

Fig. 5. Spectral response curve of fish chromoreceptor given by continuous line. Broken line shows narrowest conceivable computed spectral sensitivity curve of fish chromoreceptors. Heavy line indicates modulator curve obtained by *Donner* (1953) on pigeon.

The modulator curves described by *Donner* are exclusively obtained on pure "on-off" responses of the retinal ganglion cells, whereas the spectral response curves of the cones correspond to steady receptor potentials continuing as long as the light stimulus lasted. Only achromatic light, or spectral stimuli, close to the neutral region between the two opposite spectral response curve maxima, evoked receptor potentials corresponding to "on-off" responses.

Looking at Fig. 3, it appears that the neutral point (np_1) of the R-G spectral response curve, which is situated in the middle between the opposite R and G maxima, rather well coincides with the maximum of the modulator curve to the extreme right. The neutral point of the R-G spectral response curve is shifted towards the short wave end of the spectrum by about 10 mμ as compared with the modulator curve, which value actually agrees with the shift existing between the maximum of the L cone of fish and the photopic dominator of the pigeon (Fig. 4).

Using pure spectral lights, *Lashley* (1916) was able in "Dressur" experiments to train his game bantam cocks to discriminate red 650 mμ from other colored and white lights of any brightness. Red light of the wavelength 650 mμ is rather close to the "red" maximum of the spectral response curve recorded from single cones of fish. It is, however, more than 50 mμ away from the maximum of the "reddest" narrow band modulator curve obtained by *Donner* on pigeon. Further, considering (1) the narrowness of the modulator curves, (2) the fact that they were obtained on pure "on-off" responses, and (3) that the color signals mediating midget ganglion cells (7—8 μ) are too small to be reached with the microelectrode technique used, there seems to

be little reason to assume that the modulator curves should correspond to the maxima of spectral response curves of the chromoreceptors.

The striking agreement between the modulator maximum and the neutral point of the R-G curve, together with the fact that this neutral region of the spectrum corresponds to "on-off" responses of the receptor potentials, makes it more likely to consider the modulator curve (to the right, Fig. 3 b) as reflecting the neutral region (np_1) of the spectral response curve.

Since there is no agreement either between the two remaining modulator curve maxima and the crests of the B, G and Y spectral response curves, there are reasons to search for other possible neutral points which correspond to these modulator curves. In the recorded spectral response curve of the Y-B receptor in fish, due to the low amplitude of the Y-response, the neutral point was rather close that of the R-G cone pair. In correspondence with the R-G curve, it is assumed that the actual neutral point for the Y-B cone pair is situated in the middle between the Y and B maxima. This point np_2, as appears from Fig. 3, agrees with the maximum of the middle modulator curve, the difference again being about 10 mμ! Similarly, the maximum of the third modulator curve (to the left) coincides with a 10 mμ shift (!) with an assumed neutral point between the maxima of the G and R_2 spectral response curves.

From these findings to conclude, the three neutral points of the fish and frog seem to be situated approximately in the corresponding regions of the spectrum, whereas those of the bird are shifted by about 10 mμ towards the long wave end of the spectrum; a value which agrees with the difference between the maximum of the photopic dominator of the pigeon and the maximum of the L type cone of fish. The agreement of all these data seems to be too good just to be a coincidence. A further support for the correctness of these findings appears from the studies on color blindness, which show the existence of three neutral points in the human spectrum too, approximately corresponding to the three neutral points constructed above (the red-green blind having one, and the yellow-blue blind showing additionally two, *e.g. Judd* 1951 and part VI, this suppl.).

It seems to be a substantially correct view (*Polyak* 1941) that the chromaticity signals are carried by the midget type of ganglion cell (or corresponding), the spikes of which being unobtainable with

microelectrodes hitherto used, and that information concerning luminosity converges from a large number of cones and rods on the diffuse types of ganglion cell. Further, it seems probable that certain large and medium sized diffuse ganglion cells transmit the "on-off" signals from the chromoreceptors.

A comparison of the observations made on single cones of fish with the findings obtained on retinal ganglion cells of different vertebrates seems to prove that the organization of the retina of the diurnal vertebrates is based essentially on identical principles.

On the basis of the results hitherto achieved by recording the spike activity of the retinal neurons (dominators and modulators) it appears to be impossible to draw any conclusions regarding the chromoreceptors. It appears also unjustified to conclude from the findings obtained by the retinal spike method that the photopic luminosity mechanism (dominators) should be built up of the different chromoreceptors.

In agreement with the views presented above, *Walls* (1953) seemed to be right in his criticism of *Granit*'s work, when he concludingly stated: "In short, *Granit* is convinced that he is dealing with a color-vision mechanism, whereas I have been convinced for a decade that he is not." The modulator curves obtained on frog (*Granit* & *Svaetichin* 1939) and on pigeon (*Donner* 1953) apparently do not reflect the chromaticity signals, — but rather possibly those for achromaticity. This is also in agreement with the opinion held by *Walls* (1953) since, concerning the modulator curves, he wrote (p. 84) that they "may have a very important physiological significance, but not in relation to the animal's color vision."

The attempts to combine the results obtained by different methods might be of use in future research which will give "the final general solution of the problem".

REFERENCES

Bailey, F. W. & C. F. C. Riley, Trans. Roy. Canad. Inst. 1931. *18.* 47.

Barlow, H. B., J. Physiol. 1953. *119.* 58.

— J. Physiol. 1953. *119.* 69.

Bernhard, C. G., Acta Physiol. Scand. 1941. *3.* 131.

Blässer, A., Zool. Jb. Abt. Physiol. 1926. *43*. 69.
Brossa, A. & A. Kohlrausch, Arch. Anat. Physiol. 1913. 449.
Chaffe, E. L. & A. Hampson, J. Optic. Soc. America. 1924. *9*. 1.
Cohn, R., J. Neurophysiol. 1956. *19*. 416.
Detweiler, S. R., Vertebrate Photoreceptors. Exper. Biol. Monographs. 1943.
Ditchburn, R. W. & B. L. Ginsborg, Nature. 1952. *170*. 36.
— J. Physiol. 1953. *119*. 1.
Donner, K. O., J. Physiol. 1953. *122*. 524.
Engström, K., Personal communication 1956.
Graham, C. H., E. H. Kemp & L. A. Riggs, J. Gen. Physiol. 1935. *13*. 275.
Graham, C. H. & L. A. Riggs, J. Gen. Physiol. 1935. *12*. 279.
Granit, R., Nature, 1937. *140*. 972.
Granit, R. & A. Munsterhjelm, J. Physiol. 1937. *88*. 436.
Granit, R. & C. M. Wrede, J. Physiol. 1937. *89*. 239.
Granit, R. & G. Svaetichin, Uppsala Läkaref. förh. 1939. *65*. 161.
Granit, R., Acta Physiol, Scand. 1940. *1*. 386.
— Acta Physiol. Scand. 1941. *2*. 93.
— Acta Physiol. Scand. 1941. *2*. 334.
— Acta Physiol. Scand. 1942. *3*. 318.
— Acta Physiol. Scand. 1942. *4*. 118.
— Acta Physiol. Scand. 1943. *14*. 108.
— Nature. 1943. *151*. 11.
— Acta Physiol. Scand. 1943. *5*. 219.
— J. Neurophysiol. 1945. *8*. 194.
— Sensory Mechanisms of the Retina. Oxford Univ. Press. 1947.
— Ergebn. Physiol. 1950. *46*. 31.
— Receptors and Sensory Perception. Yale Univ. Press. 1955.
Grundfest, H., J. Gen. Physiol. 1931. *15*. 307.
— J. Gen. Physiol. 1932. *15*. 507.
Hamilton, W. F. & T. B. Coleman, J. Comp. Psychol. 1933. *15*. 183.
Hartline, H. K. A., Amer. J. Physiol. 1938. *121*. 400.
— Amer. J. Physiol. 1940. *130*. 690.
Himstedt, F. & W. A. Nagel, Ber. naturf. Ges. 1901. *11*. 153.
Honigmann, H., Pflüg. Arch. ges. Physiol. 1921. *189*. 1.
Ingvar, D. H., Abstracts of XXth International Physiological Congress. 1956. 459.

Judd, D. B., Hdb. Exp. Physiol., ed. Stevens. New York 1951. 811.
Kuffler, S. W., J. Neurophysiol. 1953. *16.* 37.
Lashley, K. S., J. Animal Behavior. 1916. *6.* 1.
Meyer, D. R., R. C. Miles & P. Ratoosh, J. Neurophysiol. 1954. *17.* 298.
Ottoson, D. & G. Svaetichin, Acta Physiol. Scand. 1953. *29.* Suppl. 106. 538.
Piper, H., Arch. Anat. Physiol. 1904. 453.
— Arch. Anat. Physiol. 1905. 153.
Plath, M., Z. vergl. Physiol. 1935. *22.* 691.
Polyak, S. L., The Retina. Chicago Univ. Press. 1941.
— Docum. Ophtal. 1949. *3.* 24.
Prince, J. H., Comparative Anatomy of the Eye. Springfield 1956.
Ramón y Cajal, S., Die Retina der Wirbeltiere. Wiesbaden 1894.
— La rétine des vertébrés. Trav. Lab. Rech. biol. 1933. Suppl. 28. 1.
Ratliff, F., J. Exptl. Psychol. 1952. *43.* 163.
Révész, G., Z. Psychol. Physiol. Sinnesorg. 1921. *87.* 130.
Riggs, L. A., F. Ratliff, J. C. Cornsweet & T. N. Cornsweet, J. Optic Soc. America. 1953. *43.* 495.
Riggs, L. A., J. C. Armington & F. Ratliff, J. Optic Soc. America. 1954. *44.* 315.
Rushton, W. A. H., Nature. 1949. *164.* 743.
— J. Physiol. 1950. *111.* 26 P.
— Brit. Med. Bull. 1953. *9.* 68.
Svaetichin, G., Acta Physiol. Scand. 1948. *16.* Suppl. 53. 61.
— Acta Physiol. Scand. 1951. *24.* Suppl. 86. 5.
— Acta Physiol. Scand. 1951. *24.* Suppl. 86. 23.
— Acta Physiol. Scand. 1953. *29.* Suppl. 106. 601.
Tomita, T., Jap. J. Physiol. 1950. *1.* 110.
Tomita, T. & A. Funaishi, J. Neurophysiol. 1952. *15.* 75.
Wald, G., Ann Rev. Biochem. 1953. *22.* 497.
Walls, G., The Vertebrate Eye and its Adaptive Radiation. Michigan 1942.
— Univ. Calif. Publ. Physiol. 1953. *9.* 1.
Watson, J. B., Pap. Dept. Mar. Biol. Carn. Inst. Wash. 1915. *7.* 87.
Wojtusiak, R. J., Z. vergl. Physiol. 1932. *18.* 393.
Wright, W. D., J. Optic. Soc. America. 1952. *42.* 509.

VI. ASPECTS ON HUMAN PHOTORECEPTOR MECHANISMS

BY

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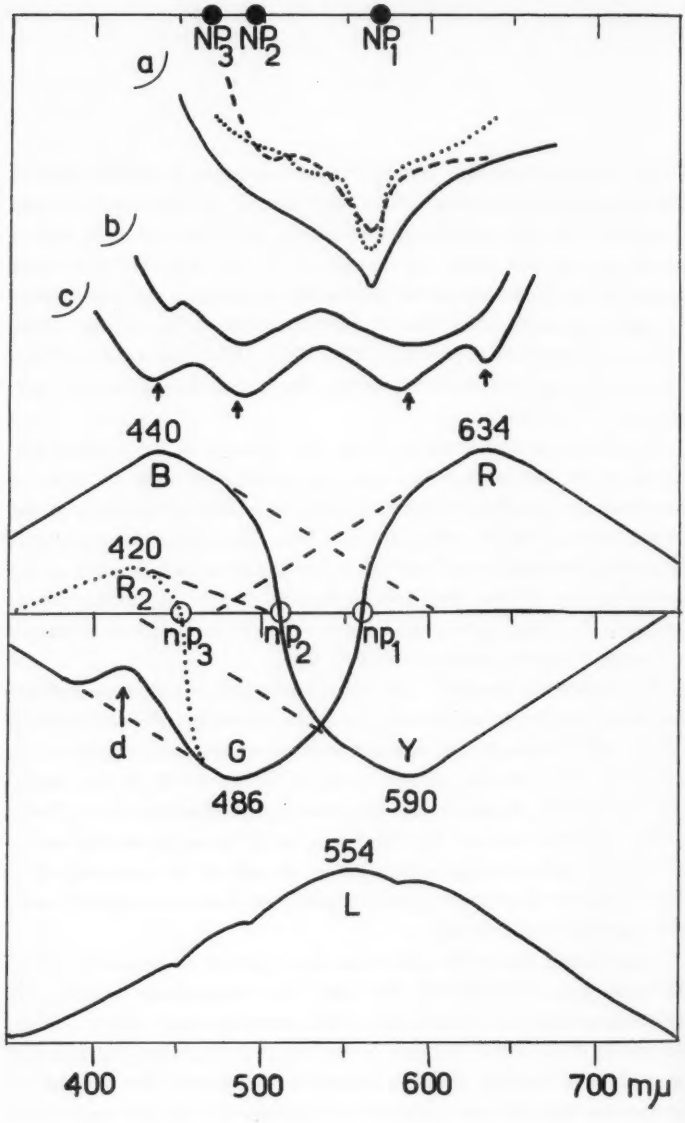
From studies on spectral response curves obtained on single cones of the fish retina (*Svaetichin* 1953 a, and parts I, II, this suppl.) it was concluded that the peripheral mechanism for color vision in fish is based on the twin cones (R-G and Y-B). In fish the twin cones appear to be chromoreceptors functioning in pairs, in accordance with *Hering*'s opponent color theory (*Hering* 1876, 1925, *Müller* 1896, 1897, *v. Tschermak-Seysenegg* 1929, 1947, 1952, and *Linksz* 1952). The single type of L cone represents the retinal mechanism for perception of luminosity.

An attempt is now made to relate the findings on the receptor mechanisms in fish to human vision psychophysical data in order to ascertain the possible existence of similar general principles for the visual mechanisms in both man and fish. The information obtained from the investigations of the fish cones has been used as a key in the interpretation of data from psychophysical studies on human vision; and actually visual perception in man seems to be based on a pattern of receptor signals similar to that in fish.

The commonly accepted view is that the human receptor mechanisms are built on a trichromatic basis (*e.g.* the survey by *Müller-Limmroth* 1956), and the psychophysical phenomena on which *Hering* based his opponent color theory, should occur at higher levels in the central nervous system. However, the twin wavelength discriminator (*Linksz* 1952), the twin cone of the fish retina being its morphological correspondence, seems a reasonable solution, already at the cone level offering a delicate chromaticity discrimination mechanism compatible with the opponent color theory.

Considering that color vision has been proved to be similar in all essential parts in both fish and man (four maxima on the hue discrimination curve, a closed color circle, complementary colors; see the survey by *Walls* 1942, and part II, this suppl.), it can generally be said that all facts favoring *Hering*'s opponent color theory also support the suggestion that the photoreceptor mechanisms in both fish and human are built on the same general principles.


NP3
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a
b
c
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634
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590
554
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400
500
600
700 mμ

Hue Discrimination Curve.

On the basis of the spectral response curves obtained on fish cones the scheme in Fig. 1 has been drawn. The maxima of the curves (Fig. 1) have been shifted by 20 mμ towards the short wave end of the spectrum, as compared to those of the fish curves (*cf.* Figs. 1, 3 and 9, part II, this suppl.). In the diagram in Fig. 1 human hue discrimination curves have been drawn; (b) is a mean curve redrawn from *Wright* (1946, Fig. 95) and the curve (c) has been redrawn from *Jones* (1917, and *Crozier* 1950).

It appears that the by 20 mμ shifted maximum of the spectral response curve of the L cone of fish coincides with the crest of the human photopic luminosity curve. Further, it is seen that the by 20 mμ shifted maxima of the B, G, Y and R spectral response curves of fish (Fig. 1 c, arrows below the human hue discrimination curve) also agree well with the hue discrimination maxima (*Jones's* curve, maxima for discrimination downwards).

In a conventional hue discrimination curve (*e.g. Wright* 1946) the just noticeable difference in wavelength is plotted against the wavelengths at which judgement has been made. All authors agree in respect to the pronounced reduction of the hue discrimination ability in the middle part of the spectrum (Fig. 1 b, c) corresponding to the middle region between the G and Y receptor maxima. Actually, this decrease of the hue discrimination ability strikingly corresponds to the low sensitivity of the color receptors in the region between the G and Y maxima. According to the diagram in Fig. 1, the distance between the maxima of G and Y receptors is 104 mμ, which is about twice the distance between the B, G and Y, R receptors, which is 46 and 44 mμ respectively.

Most authors have obtained another minimum (upwards in Fig. 1 b,

Fig. 1. Schematically drawn spectral response curves of fish luminosity receptors (L) and chromoreceptors (B, G, Y and $R+R_2$), all shifted by 20 mμ towards the short wave end of the spectrum. At (a) spectral saturation discrimination curves obtained on man by *Chapanis* 1944 (broken and dotted lines) and by *Wright* 1946 (continuous line). Hue discrimination curves obtained on man (discrimination optima downwards) redrawn from *Wright* 1946 (b), *Jones* 1917 and *Crozier* 1950 (c). Arrows below curve (c) indicate maxima of fish chromoreceptors shifted by 20 mμ towards the short wave end of the spectrum. $NP_{1\text{-}3}$ and filled circles on top of diagram give neutral regions of dichromats (man). $np_{1\text{-}3}$ and open circles denote neutral points of fish chromoreceptors (shifted by 20 mμ).

c) of the hue discrimination curve in the region between the B and G receptor maxima, whereas several authors *e.g. Wright* & *Pitt* (1934, see the reviews by *Wright* 1946 and *Hartridge* 1950) deny the existence of a third minimum in the region between the Y and R receptor maxima.

For light stimuli of high intensities the receptor potentials in the middle region between the maxima of the spectral response curves (R-G and Y-B in Fig. 1) were of sufficient amplitudes, whereas low intensity test stimuli evoked receptor responses which reached an amplitude value close to zero not far from the maximum of the spectral response curve. The low receptor sensitivity in the region between two adjacent chromoreceptor maxima seems to be the reason why, with low intensity test stimuli, the hue discrimination is reduced between B & G, G & Y, and Y & R, respectively. This possibly also explains the different results obtained by *Wright* & *Pitt*, by *Jones*, and by *Laurens* & *Hamilton* 1923. In determining the hue discrimination curve, *Wright* & *Pitt* used test lights of an intensity of 70 photons, whereas the light intensity in the corresponding measurements by *Laurens* & *Hamilton* was only 4 photons. *Wright* & *Pitt* used the strongest monochromatic light they could obtain, and this was about four times stronger in the red than in the blue part of the spectrum.

On the basis of the above presented interpretation it is conceivable that the optima for hue discrimination coincide with the maxima of the color receptors.

Hue Perception.

Twin and single cones of the kind occurring in the fish retina have been drawn, together with their corresponding characteristic responses, in the scheme in Fig. 2 a, b and c. In Fig. 2 d the most common mosaic pattern of the fish cones is shown together with tentative indications of the receptor types, as revealed by microelectrode studies. The "on-off" response proved to be caused by the latency differences between the opposite receptor responses as indicated in the diagram in Fig. 2. In a twin cone system the opponent receptor responses are subtracted from each other, the polarity and amplitude of the remainder response (if any) determine which of the two opponent colors is signalled.

As appears from the scheme in Fig. 2, the L type of single cone gives one and the same monophasic hyperpolarization response to all

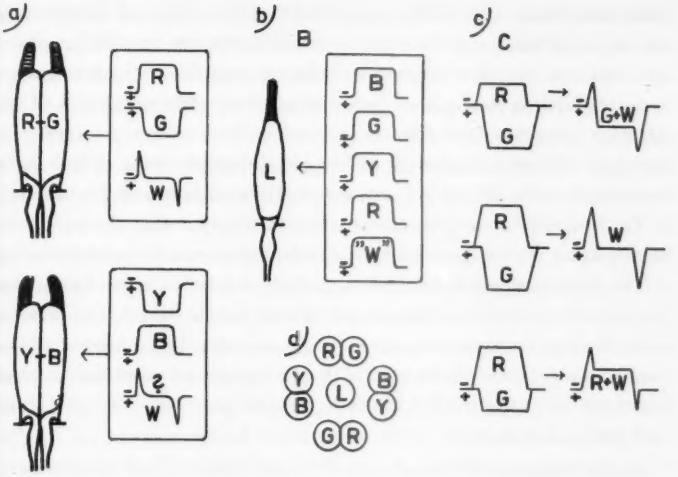

Fig. 2. Twin chromoreceptors R-G and Y-B of fish at (a) and luminosity receptor L at (b) drawn together with the characteristic receptor responses. Subtraction of opposite receptor potentials shown at (c). Common cone pattern of fish and tentative indication of cone types shown at (d).

the different qualities of light stimuli, whereas the R-G and Y-B twin cones are able to produce different signals for the colors red, green, yellow and blue (and possibly also for achromacy).

From the characteristics of the receptor potentials of the R-G twin cone it follows that this twin cone is able to signal either red or green, but never both simultaneously, and the circumstances are analogous for the Y-B twin (Figs. 1, 2). On this basis a signal for any other hue than the unique hues—blue, green, yellow and red, is composed of maximally two different receptor responses (B+G, G+Y, Y+R, or B+R). Further, an intermediate hue, being a mixture between opponent color pairs, cannot exist, since in this case both members of the same twin cone are stimulated and the opponent receptor responses cancel each other. Thus, a hue is apparently signalled by maximally two different receptors simultaneously (Figs. 1, 2). This is in agreement with the fact that for humans there are no hues perceived as combinations of the opponent colors (neither reddish-greens nor

yellowish-blues). It is further suggested that the quality of the perceived hue depends solely on the *relation* (!) between the amplitudes of the two different receptor responses. Since, as suggested, the hue quality is determined on the basis of the relation between the amplitudes of two receptor responses, this relation is not disturbed when the intensity of the light stimulus is altered within physiological limits, which is in accordance with the color constancy at different levels of illumination.

The *Brücke-Bezold* phenomenon favors the view that the yellow receptor has a higher threshold than the other chromoreceptors in man.

The twin cone mechanism is apparently favorable, considering that the central nervous structures never have to handle the relation between more than two receptor responses in one moment. There might be some connection between the reduction to two signals only and the fact that a neuron can be governed by two opposite processes—the excitatory and the inhibitory.

In accordance with the above mentioned, color blind (dichromats) would be able to perceive two unique hues but no intermediary hues, since they possess only the one type of twin cone, combinations between opponent colors being excluded. According to the interpretation of *Hecht* & *Shlaer* (1935), wavelength discrimination for dichromats is entirely determined by saturation differences in the spectrum, which is in good agreement with the views presented above.

A dip seen in the course of the decay of the G spectral response curve obtained on fish cones indicates the existence of an R_2 maximum in fish (part II, this suppl.). The presence of a submaximum R_2 of the spectral response curve of the R cone also in humans, is supported by color mixing experiments and by observations on color blind subjects.

The hue discrimination curve shows a steeper decay in the red end of the spectrum (Fig. 1 c) than in the violet one, favoring the existence of the R_2 submaximum in this region. The submaximum R_2 would also explain why the short wave end of the spectrum appears violet. The suggested fluorescence of the eye media causing the reddishness of the short wave end of the spectrum does not seem to have any experimental support and would be obsolete with the above given explanation.

Neutral points.

The Y spectral response curve, which in the recordings from the fish cones always showed a lower amplitude than the other ones (part II,

this suppl.) has been drawn in the diagram in Fig. 1 with the same height as the other curves. The neutral point (np_2) between the two opponent maxima of the spectral response curve has, in analogy with the neutral point of the R-G curve (np_1), been placed midway between the maxima in the Y-B curve too. A third neutral point (np_3) is suggested to exist midway between the opponent G and R_2 maxima, R_2 being supposed to be the submaximum of the R spectral response curve (part II, this suppl.). It is worth mentioning that the maxima of the modulator curves obtained on frog and pigeon on the basis of recordings from retinal ganglion cells, well agree with the above mentioned neutral points (part V, this suppl.).

The neutral regions observed in studies on color blind subjects have been indicated on the top of Fig. 1 by the filled circles (*cf.* Kardinalpunkte *e.g. v. Tschermak-Seysenegg* 1947). NP_1 (570—580 mμ) and NP_3 (470 mμ) correspond to the parts of the spectrum the yellow-blue blind sees neutral, and the NP_2 (495 mμ) indicates the region which is neutral for the red-green blind (*e.g. Judd* 1951). These points roughly correspond to the neutral points ($np_{1\text{-}3}$) of the spectral response curves obtained from fish cones.

Achromacy and Desaturation.

Above in Fig. 1 spectral saturation discrimination curves of humans have been drawn (continuous line at "a" is a mean curve redrawn from Fig. 93 in *Wright* 1946, and dotted and broken lines represent curves redrawn from Fig. 3 in *Chapanis* 1944). It appears that the region for maximal desaturation (downwards Fig. 1 a) agrees approximately with the neutral point np_1 and also with the maximum of the spectral response curve of the L cone. In addition to this, the saturation minimum is also close to the maximum of the Y spectral response curve. Some of the saturation discrimination curves show additional humps in the short wave end of the spectrum, possibly corresponding to the neutral point np_2?

It proved to be difficult to determine spectral saturation discrimination curves. Desaturation is defined by the proportion of achromatic (white) light in a color mixture. The judgement of the degree of desaturation (or saturation) needs more concentration and is not so simple and straightforward as that of perception of hue, which favors the view that there is no special receptor signal for desaturation proper.

Hence, it might be suggested that the degree of desaturation is judged by the higher nervous centres on the basis of the amplitude difference between the response of the L type of cone signalling the total amount of light (luminosity) and the chromatic responses mediated by the twin receptors.

It is well known that the yellow spectral color is the most desaturated, but also the most luminous one, and further, blue and red are spectral colors more saturated than green. These facts are easily explained on the basis of the proposed mechanisms for cone vision (Figs. 1, 3).

The "on-off" responses evoked when the twin cones were stimulated by achromatic light or by spectral lights of wavelengths close to the neutral points of the spectral response curves (part. II, this suppl.) could, of course, constitute some kind of additional receptor signals for achromacy. This view is supported by the following observations: 1) the receptor potential evoked by an achromatic stimulus of the twin cones is actually an "on-off" response, 2) the modulator curves obtained by *Donner* (1953, *cf.* part V, this suppl.) coincide with the neutral points $np_{1\text{-}3}$ and were all obtained on ganglion cells producing "on-off" spike patterns; thus, the "on-off" responses of the twin discriminators are signalled to the higher centers and seem to be of functional significance, 3) the neutral points $np_{1\text{-}3}$ agree approximately with the neutral regions ($NP_{1\text{-}3}$, Fig. 1) of color blind subjects, and 4) the neutral point np_3 is close to the spectral region showing maximal desaturation.

The yellow perceived when using a monochromatic spectral light, is more saturated than the yellow produced by mixing spectral red and green. In the latter case the R-G cone pair is subjected to a stronger light stimulus (the opposite R and G responses cancelling each other) than when spectral yellow is used, which is a light stimulus close to the neutral point np_1 of the R-G spectral response curve (Fig. 1, 3). This favors the view that some additional signal for desaturation (achromacy, white) is delivered by the twin cones.

In order to produce "on-off" responses, the light has necessarily to be switched on and off. However, looking continuously against an evenly illuminated colored wall, it is possible to perceive both hue and saturation. Considering this fact, the "on-off" receptor signals are possibly not important for the perception of desaturation and achromacy; for contrast phenomena they might be more important.

Photopic Luminosity Receptor.

The existence of a mechanism for luminosity separate from the chromoreceptor mechanisms in humans, is indicated by several facts. The shift of the luminosity maximum from yellow in photopic to green in scotopic conditions (*Purkinje* phenomenon) is hard to explain on the basis of receptor mechanisms common for both chromaticity and luminosity. On this basis it is also difficult to see why the photopic luminosity curves for color blind subjects are practically identical with

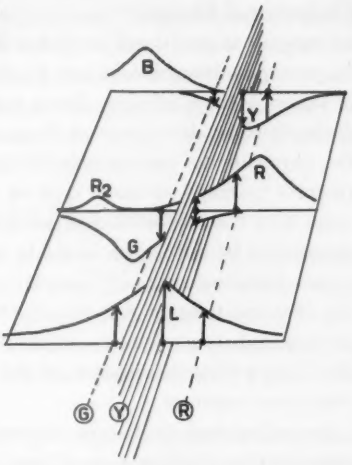

Fig. 3. Diagram of spectral response curves. Lines at (G), (Y), and (R) indicate bundles of spectral lights.

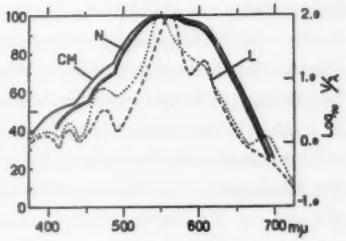

Fig. 4. Human photopic luminosity curves (Log V_λ) obtained by *Wright* (1952) on normal subject (N, heavy line) and on cone monochromat (CM, continuous line). Spectral response curves of L type of fish cone (L, broken and dotted lines) shifted by 20 mμ towards the short wave end of the spectrum.

those of normals. Totally color blind cone monochromats show a photopic luminosity curve in detail identical with that of normal subjects (*Wright* 1952), even displaying the three humps which have been considered to indicate chromoreceptor mechanisms.

In Fig. 4 photopic luminosity curves (redrawn from Figs. 3 and 14 in *Wright* 1952) obtained on a normal subject (heavy line) and on a cone monochromat (continuous line) are presented together with spectral response curves of the L type (broken and dotted lines) of fish cones, the latter shifted 20 mμ towards the short wave end of the spectrum. It is seen that the humps on the human photopic luminosity curve approximately agree with the submaxima seen on the L type of spectral response curve.

Flicker and Fusion.

Investigations performed on flicker fusion have proved that the fusion frequency is determined solely by the luminosity and is independent of the wavelength of light. These observations are easily explained on the basis of the above mentioned separation between the luminosity and the chromaticity mechanisms. If the assumption is made that the receptor potential of the L type of cone has shorter rise and decay times than that of the twin chromoreceptors, the frequency for fusion determined by the L cone would be higher than the fusion frequencies of the chromoreceptors (*cf.* part III, this suppl.). The time constants of the different receptor responses in fish have not yet been thoroughly investigated. Several observations showed that the time constants of the L type of cone response of fish were shorter than those of the twin cone responses.

Assuming that the L type of cone of the human retina shows the highest fusion frequency, the L cone would then determine the maximal fusion frequency and hence, the fusion frequency would depend solely on the relative luminosity of the spectral colors. This is apparently the reason why the curves showing the relation of critical frequency to Log intensity of light, are identical and independent of the wavelengths of light (*Porter* 1898, 1902, *Hecht* & *Shlaer* 1936).

The mechanism for heterochromatic flicker photometry, in which the luminosity of different spectral light is compared, is easily explained on the basis of the above assumption. Concerning heterochromatic flicker photometry, *Duke-Elder* (1942, p. 875) writes:— "The two lights to be compared are alternated with each other. When this is done at a certain speed of rotation the sensation of colour-flicker disappears, but if the two lights differ in luminosity, brightness-flicker is still perceptible and does not disappear until the speed is increased. *There is thus a speed at which brightness can be completely dissociated from the influence of colour.* The two lights are thus alternated at this speed so that brightness-flicker only is present, and their relative intensity is adjusted so that no flicker is perceived; the adjustment being such that the slightest change in intensity of either light causes it to appear again. At this point they must be equally bright."

When the relative luminous efficiency for flicker fusion of different spectral lights is plotted against the wavelength, we get the "standard photopic luminosity curve", a curve which in fact is identical with the

human photopic luminosity curve, the latter being obtained by plotting the reciprocal value of threshold energy against the wavelength. Both curves are apparently based on the properties of the L type of cone.

The suggestion made above that the human photopic luminosity mechanism depends on the existence of the L type of cone, is strongly supported by the above mentioned findings.

Intensity Discrimination and Visual Acuity.

Curves obtained in cone visual function measurements on man, relating brightness discrimination, visual acuity, and critical fusion frequency to Log intensity of the light stimulus, show a sigmoid course similar to the curves showing the relation between the receptor response amplitude and the Log intensity of light (*Svaetichin* 1953 a, part I, this suppl.).

Several theories have been developed in order to explain these visual phenomena; some of them are on a photochemical basis, whereas other theories are founded on the assumption of a statistical variation of the thresholds of the receptors (see the survey by *Jahn* 1950).

The fish cones did not show signs of adaptation when stimulated by moderate light stimuli. The thresholds and the receptor response amplitudes of the different types of cones were all about equal (except Y). Assuming that the properties of human cones are similar to those of the fish, the theories (*e.g. Hecht* 1927, 1934) based on the suggested threshold difference, cannot be true.

It appears reasonable to assume that the sigmoid course of the receptor response amplitude—Log intensity relation curve, is reflected in the contour of the curves relating brightness discrimination, visual acuity and critical fusion frequency to Log intensity of light stimulus.

Visual acuity varies with the wavelength of the illuminating light and is directly related to the luminuous efficiency of the particular spectral region. This observation favors the view that visual acuity is mainly determined by the L type of cone, the chromoreceptors additionally offering "on-off" responses corresponding to contrast effects at border lines.

White, Gray and Black.

It appeared that the fish cones did not show signs of adaptation when subjected to a light stimulus of moderate intensity (*Svaetichin* 1953

and part II, this suppl.). It was suggested above that the difference between the total amount of radiation energy signalled by the L type of cone and the chromatic part signalled by the twin discriminators constitutes the base on which the degree of desaturation is judged. Further, it appeared reasonable to assume that the perception of black is based on the absence of the L response or even more on the inhibition of that response (*cf.* part II, this suppl. and below). Luminosity proper is signalled by the L type of cone and, from the receptor response to conclude, this type of cone is not able to differentiate any qualities of light.

The question arises, of course, on which receptor signals the perception of gray and white is based. The only types of receptors which are able to discriminate between chromatic and achromatic radiations are the twin cones. On the other hand, it is well known that the level of illumination and the contrast effect of the surroundings have a decisive influence on our perception of black and white. The general level of illumination seems to be signalled by the L type of cone, which has a range of about three Log units in fish (possibly four in humans, *cf.* part I, II, and III, this suppl.) and this level of illumination influences our perception of white, gray and black.

It might be suggested that the "on-off" response of the twin cones signals white, and that this signal is evaluated and compared with the information given by the L type of cone. The "on-off" responses have a chance to be evoked at the borders between fields of different illuminations and hues and might be suggested as being responsible for simultaneous contrast effects (*cf. Hartline, Wagner* & *Ratliff* 1956).

Benham Colors.

When a wheel displaying white and black sectors is illuminated with colorless light and rotated, parts of it appear colored. This phenomenon has been interpreted to depend on differences in latency for perception of the different colors (*Pieron* 1923). Red has been shown to be the quickest one to appear, followed by green; blue being the slowest to appear and also the last to disappear. The explanation given by *Pieron* agrees with the trichromatic theory.

Starting from the observation that in fish cones the R response had a shorter latency than the G response, and the B response appeared before the Y response, I did some experiments with a sectorized wheel

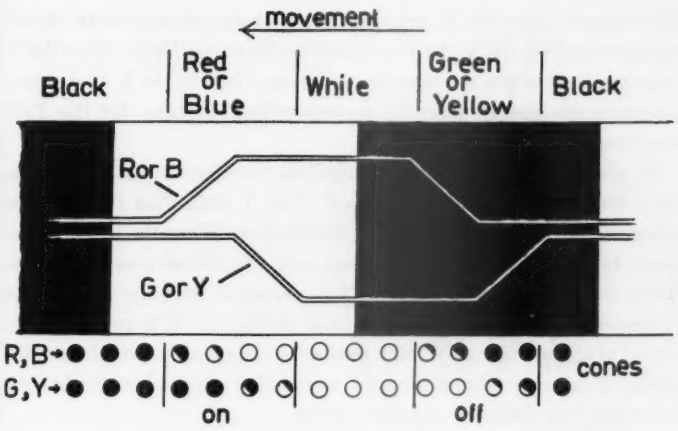

Fig. 5. Diagram illustrating suggested mechanism for development of *Benham* colors on the basis of different time constants of chromoreceptor potentials. Receptor potentials denoted by R or B and by G or Y in the middle of black and white sectors. On top of diagram the locations of the perceived chromatic effects are given. At bottom of figure chromoreceptors in different states of excitation are indicated by open and filled circles.

of the kind illustrated in Fig. 40, p. 175 in *Helmholtz* 1911. With a speed above the fusion frequency the wheel appeared totally achromatic. When the speed of rotation was reduced, there appeared red and green colors at a certain frequency of rotation. At a still lower frequency the colors changed to blue and yellow. At a frequency between the above frequencies red and green colors appeared on the peripheral ring (which moves with a higher speed), whereas the colors blue and yellow were seen simultaneously on the central ring (which moves with a lower speed). At a certain rotation frequency a mixture of red+blue and green+yellow was seen in one ring.

When a white field began to appear in the field of vision, the red (or blue, or a mixture of them) was seen first, followed by green (or yellow or a mixture of them), as is shown in the diagram in Fig. 5. Red and green were well observed with the central vision, whereas blue and yellow appeared optimally with the peripheral vision.

The phenomena described above are easily explained on the basis of the existence of R-G and Y-B twin receptor mechanisms in human,

if we assume that the R response has a shorter latency than the G response, and similarly, the B response has a shorter latency than the Y response, further that the latencies and decay times of the R-G receptor responses are shorter than the corresponding constants for the Y-B responses.

In accordance with these assumptions, the diagram in Fig. 5 has been drawn, the letters R or B and G or Y indicating the opposite receptor potentials in the center of the diagram. When the image of the black and white sectors is sweeping over the retinal cone layer, the white front produces successive "on" responses and the black front successive "off" responses (see bottom of Fig. 5). It is reasonable to expect that the "on" and "off" responses respectively, which are dispersed in time, produce a temporal summation effect which evokes the corresponding different hue perceptions.

Phosphenes.

Developing *Hering*'s theory for vision, *Müller* (1897 a) supposed that the opponent retinal processes were accompanied by potentials of opposite polarity. He says, for instance, that in the case of the R-process being accompanied by a negative electrical wave in the optic nerve, the G-process would necessarily be followed by a positive one. In experiments with D. C. polarization of the eye it has been shown (*e.g. Müller* 1897 b, *Schwartz* 1890, see further the survey by *Hartridge* 1950) that with an anode on the eyelid a phosphene of bright violet or reddish-blue was seen, whereas the phosphene was dark yellow-green, when the electrical current was of opposite polarity. Red-green blind subjects observed a blue phosphene with the anode on the eyelid and a yellow one with the cathode (*Müller* 1897 b). With the anode on the eyelid a current just above the threshold produced luminosity sensation proper, whereas a current of opposite polarity gave the sensation of black; in a dark room the blackness was accentuated (*Schwartz* 1890).

Looking at the diagrams in Figs. 1, 2 and 6, it appears that the L, B and R receptor responses are opposite to those of the Y and G receptors, which actually would explain the different effects on the phosphenes of the plus and minus currents. The accentuation of the sensation of black could possibly be explained by some inhibitory effect corresponding to a current of polarity opposite to that caused by the L receptor response (*cf. Adams* 1923, and part II, this suppl.).

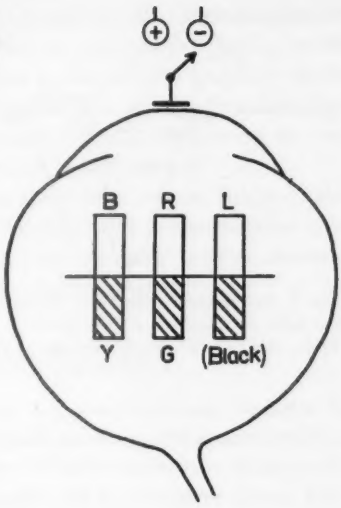

Fig. 6. Polarity of cone responses in human retina suggested on the basis of electrical phosphenes (*cf. Adams* 1923).

Thus, the observations made on the electrical phosphenes are readily explained on the basis of the suggested opponent receptor responses.

The site of action of the electrical currents producing the phosphenes, seems to be in the neurons, which are synaptically connected to the receptors. This view is further supported by experiments in which the human eye has been stimulated by A. C. current. The intermittent phosphenes thereby evoked show a maximal fusion frequency of about 90 c/s (*Rohracher* 1935, *Schwartz* 1938), which is considerably higher than the maximal fusion frequency for light flicker (50 c/s). The maximal fusion frequency of the light stimuli is apparently determined by the time constants of the action potential of the L type of cones, whereas the maximal fusion frequency of the phosphenes seems to be determined by the properties of the bipolar cells.

In experiments in which I stimulated the frog retina by electrical currents, it proved impossible electrically to evoke an ERG response. However, it was possible to polarize the retina and thereby either increase or decrease (depending on polarity) an ERG response to a light stimulus. Thus, the receptor membranes appear to be polarizable but not excitable by electrical currents.

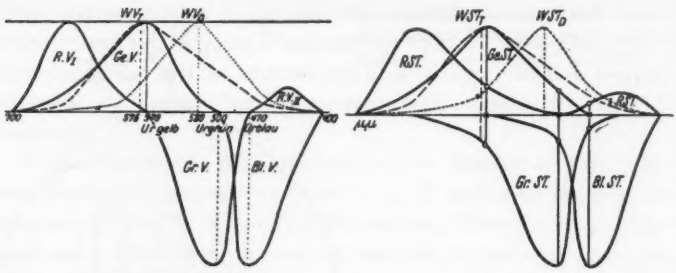

Fig. 7. *Hering* theory diagrams. To the left, stimulus value (Valenz) curves. To the right, suggested absorption curves of photopigments (*v. Tschermak-Seysenegg*, 1947). Notice blue end of spectrum to the right.

The observations made on phosphenes support an electrical theory for synaptic excitation, at least for the cone synapses; both the cathodal and anodal excitation of the retinal neurons being possible (parts II and VII, this suppl.).

Hering Theory Diagrams.

It is interesting to notice the striking resemblance between the spectral response curves recorded from the single cones of fish and the diagrams demonstrating *Hering*'s opponent color theory. In Fig. 7, two of *v. Tschermak-Seysenegg*'s diagrams are shown (1947, Figs. 31 and 51). Thus, it appears very likely that cone vision in both human and fish is based on identical basic principles.

Twin or double cones have not been described for the human retina. Certain species of *Teleost* fish have only the single type of cone, and it is not certain whether these fish species have color vision or not. Possibly a corresponding mutual counteraction effect between receptor potentials can be produced at the level of the cone synapses.

It may further be of interest to mention that insects, *e.g.* bees, are also able to see four unique hues and that the perception of intermediary hues is lacking (*v. Frisch* 1953). The ERG of insects generally shows a pronounced initial a-wave and an opposite off-effect similar to the ERG of fish and human. Receptor potentials of opposite polarity in the insect compound eye have, in fact, also been demonstrated (*Autrum* & *Gallwitz* 1951, *Svaetichin, Fernández-Morán* & *Jonasson*

1956). The receptors of the insect omatidium (for the ultra-structure of these, see *Fernández-Morán* 1956) are arranged in a regular mosaic pattern reminding of the visual cone unit of the fish retina. It appears justified to assume that the insects also possess peripheral twin chromoreceptors, whereas the central nervous mechanisms for perception of intermediary hues have not been developed.

The striking similarities between color vision of man, fish, and other animals support the view that the peripheral mechanisms for color vision in the whole animal kingdom are essentially built on the same fundamental principle, *i.e.* the twin chromoreceptors.

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REFERENCES

Adams, E. Q., Psychol. Rev. 1923. *30*. 56.
Autrum, H. & U. Gallwitz, Z. vergl. Physiol. 1951. *33*. 407.
Chapanis, A., J. Exp. Psychol. 1944. *34*. 24.
Crozier, W. J., J. Gen. Physiol. 1950. *34*. 87.
Donner, K. O., J. Physiol. 1953. *122*. 524.
Duke-Elder, W. S., Text-Book of Ophthalmology. *1*. London 1942.
Fernández-Morán, H., Nature. 1956.
Frisch, K. v., Aus Leben der Bienen. Berlin 1953.
Hartline, H. K., H. G. Wagner & F. Ratliff, J. Gen. Physiol. 1956. *39*. 651.
Hartridge, H., Recent Advances in the Physiology of Vision. London 1950.
Hecht, S., Proc. Nat. Acad. Sci. USA. 1927. *13*. 569.
— Hdb. Gen. Exp. Psychol., ed. Murchinson. Worcester 1934. 704.
Hecht, S. & S. Shlaer, J. Gen. Physiol. 1936. *19*. 965.
— J. Gen. Physiol. 1936. *20*. 83.
Helmholtz, H. v., Hdb. d. physiol. Optik. Leipzig 1911.
Hering, E., Zur Lehre vom Lichtsinn. Wien 1878. (First ed. 1876).
— Hdb. d. ges. Augenheilk. 1925. *3*. 12. 1.

Jahn, T. L., Comparative Animal Physiology, ed. Prosser. Philadelphia 1950. 381.
Jones, L. A., J. Optic. Soc. America. 1917. *1.* 63.
Judd, D. B., Hdb. Exp. Psychol., ed. Stevens. New York 1951. 811.
Laurens, H. & W. F. Hamilton, Amer. J. Physiol. 1923. *65.* 547.
Linksz, A., Physiology of the Eye. *2.* New York 1952.
Müller, G. E., Z. Psychol. u. Sinnesphysiol. 1896. *10.* 1.
— Z. Psychol. u. Sinnesphysiol. 1897. *14.* 1.
— Z. Psychol. u. Sinnesphysiol. 1897. *14.* 329.
Müller-Limmroth, H. W., Naturwiss. 1956. *43.* 337.
Pieron, H., Année Psychol. 1923. *23.* 76.
Porter, T. C., Proc. Roy. Soc. 1898. *63.* 347.
— Proc. Roy. Soc. 1902. *70.* 313.
Rohracher, H., Z. Sinnesphysiol. 1935. *66.* 164.
Schwartz, O., Arch. Psychiatr. 1890. *21.* 588.
Schwartz, F., Z. Sinnesphysiol. 1938. *67.* 227.
Svaetichin, G., Acta Physiol. Scand. 1953. *29.* Suppl. 106. 565.
Svaetichin, G., H. Fernández-Morán & R. Jonasson, Abstracts of XXth International Physiological Congress. 1956. 866.
Tschermak-Seysenegg, A. v., Hdb. d. norm. u. path. Physiol. 1929. *12/1.* 295. 833.
— Einführung in die physiologische Optik. Wien 1947.
— Introduction in Physiological Optics. Springfield 1952.
Walls, G. L., The Vertebrate Eye and its Adaptive Radiations. Michigan 1942.
Wright, W. D. & F. H. G. Pitt, Proc. Phys. Soc. 1934. *46.* 459.
Wright, W. D., Researches on Normal and Defective Colour Vision. London 1946.
— J. Optic. Soc. America. 1952. *42.* 509.

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